TROPHIC RELATIONSHIPS OF HAKE (*MERLUCCIUS CAPENSIS* CASTELNAU, 1851 AND *M. PARADOXUS* FRANCA 1960) FROM THE NORTHERN BENGUELA CURRENT ECOSYSTEM (NAMIBIA): INFERENCES FROM STABLE ISOTOPES AND FATTY ACIDS

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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ABSTRACT

Two species of hake (*Merluccius capensis* and *Merluccius paradoxus*) account for most of Namibia’s fisheries catch, and they are important secondary consumers in the Benguela Current ecosystem. Inferences on their trophic relationships have been based mainly on stomach content analyses. However, such data are limited temporally because they represent only snapshots of recent feeding, and are quantitatively biased because of variation in the digestion rates of different prey.

The principal aim of the thesis was to understand the trophic relationships of two hake species relative to each other, their known prey and top predators (demersal sharks) in the northern Benguela Current ecosystem (Namibia), using time-integrating trophic biomarkers. By using stable isotope (carbon and nitrogen) and fatty acid signatures of their muscle tissues, my overall objectives were to produce new knowledge about 1) hake ontogenic trophic relationships, 2) the contributions of different prey to hake diets, 3) hake dietary differences, and 4) some aspects of hake’s trophic relationships with demersal sharks. Tissues of hake (n=358), their potential prey (n=455), and demersal sharks (n=42) were collected between 2008 and 2012 during demersal bottom trawl surveys off Namibia, for stable isotope and fatty acid analyses.

Nitrogen isotopes ($\delta^{15}N$) showed a significant positive relationship with size in both *M. capensis* and *M. paradoxus*; however, the correlation slopes of size and $\delta^{15}N$ in the two species were significantly different, with *M. paradoxus* displaying a steeper trophic shift**.** A significant increase in carbon isotope signature ($\delta^{13}C$) with size was observed in *M. capensis* but not in *M. paradoxus*. In all size classes (except 20–29 cm) *M. capensis* had significantly enriched ^{13}C values, generally matching their respective adult shelf-slope distribution. Smaller hake from both species (20–39 cm) were trophically indistinguishable, with trophic levels (TL) of 3.3 indicating predominant zooplanktivory. The largest *M. capensis* (60–70 cm) had TLs of approximately 3.5–3.6, whereas *M. paradoxus* of the same size were slightly higher at 3.7–3.8 TL, indicating greater piscivory in the latter.

A Bayesian isotope mixing model indicated that teleosts *Synagrops microlepsis* and *Chlorophthalmus agazizi,* the shrimp *Plesionika martia*, myctophids and euphausiids dominated the diets of both hake species. The greatest contributor to the diet of *M. paradoxus* changed depending on the body size of the hake, while the diet of all size classes of *M. capensis* was dominated by myctophids. Cannibalism accounted for less than 6% and less than 4% of the diets of *M. capensis* and *M. paradoxus* respectively. Isotope-based estimates of prey dietary contributions differed from those of published accounts of gut content analyses, an indication of overestimation of the time-integrated diet by the latter.

The fatty acid (FA) profiles of the two species (*M. paradoxus and M. capensis*) and potential prey were similar to those typically abundant in marine fish. Palmitic acid (16:0) was the dominant saturated fatty acid (SFA), oleic acid (18:1n-9) was the dominant monounsaturated fatty acid (MUFA) and docosahexaenoic acid (22:6n-3) was the dominant polyunsaturated fatty acid (PUFA) in both hake species. Fatty acid (FA) profiles in the neutral storage lipids of *M. paradoxus* and *M. capensis* were significantly different, an indication of hake species' dietary resource partitioning, although some overlap was apparent. There was a significant temporal effect on hake FA compositions, reflecting temporal variations in quantity and quality of dietary resources.

Feasible contributions of hake to the diets of three demersal sharks indicated that hake are not major prey of *Centrophorus squamosus*, whose diet was dominated by crustaceans. However, there was an increased contribution of hake to the diets of *Deania* sharks. All three sharks studied (*Deania calcea, D. profundorum* and *C. squamosus*) potentially consumed upwards of 18 % (208 960 tonnes per year) of the hake biomass [2011 hake biomass estimates (1 111 196.8 tonnes)]. The two *Deania* species were isotopically indistinguishable in terms of $\delta^{15}N$, and fed on a prey composition that overlapped with that of *M. paradoxus*. $\delta^{13}C$ values indicated that hake and sharks fed on prey from similar basal resources, with *C. squamosus* being significantly different from *M. paradoxus* but not from *M. capensis*. The range of $\delta^{13}C$ and $\delta^{15}N$ indicated that the two hake species had a higher range of carbon sources as well as enhanced trophic diversity in their feeding patterns than did the three shark species.

This study is the first combined usage of stable isotope and fatty acid techniques to analyse trophic relationships of *M. capensis* and *M. paradoxus* in the northern Benguela Current (Namibia). The study contributes towards understanding of trophic interactions in Namibian waters, and can aid the implementation of a science-based ecosystem approach to fisheries management in the Northern Benguela Current region (Namibia). The extension of this analysis throughout hake distribution ranges will provide additional insights into the trophic dynamics of hake species in future, and my study represents a solid foundation from which to develop such extensions.

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DECLARATION

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author unless otherwise stated.

This thesis is dedicated to the memories of my late brother "Betuel Shalimba Iitembu" (1981-2010)

John 1:29

CHAPTER 1: GENERAL INTRODUCTION

1.1 The marine waters off Namibia

The marine waters within 1500 km of the Namibian coastline are influenced by the Benguela Current. This cold and nutrient-rich current flows between 5 ^{o}S, 12 ^{o}E off Cabinda in Angola to the Nelson Mandela Metropole (Port Elizabeth) on the south coast of South Africa at 34^{o}S, 26^{o}E [(Schell 1970; Nelson and Hutchings 1983; Hutchings *et al.* 2009) (Fig 1.1)]. The Benguela Current is bounded by two warm-water regimes, making it unique amongst the world's upwelling systems (Shannon 1985; Hutchings *et al.* 2009; Veitch *et al.* 2009). The Benguela Current is characterised by an equatorward flow and high levels of wind-driven coastal upwelling which brings nutrient-rich deep water to the surface (Nelson and Hutchings 1983; Carr and Kearns 2003). Upwelling is a dominant oceanographic process in the Benguela Current area, with other features being the leakage of warm Angola Current water from the north (Gammelsrod *et al.* 1998; West *et al.* 2004), hydrogen sulphide eruption, and red tides (Weeks *et al.* 2004; Brüchert *et al.* 2009).

The current is divided into two distinctive areas in proximity to the most active upwelling cell around Lüderitz (Shannon 1985). The northern region that is in contact with Namibian waters is characterized by intense upwelling throughout most of the year (Campillo-Campbell and Gordoa, 2004). Upwelling off Namibia is strong during the cooler months, reinforcing the seasonal effect and causing a well-defined temperature cycle (Gordoa *et al.* 2000). Seasonal warming of central and northern Namibian waters occurs during late summer and early autumn as a result of the intrusion of warm saline water of equatorial origin (Shannon *et al.* 1987). The influence of the upwelling regime is on average between 150 and 200 km wide; however, the filamentous mixing area may extend up to 625 km offshore (Campillo-Campbell and Gordoa 2004).

The Benguela Current ecosystem is one of the most biologically productive regions of the world's oceans, supporting large commercial fisheries due to its high primary productivity (Shannon 1985; Hutchings *et al.* 2009; Veitch *et al.* 2009). The energy flow pathway is dominated by primary production–zooplankton–pelagic fish–demersal fish, with pelagic and demersal fish acting as the most important planktivores and secondary consumers, respectively (Heymans and Baird 2000). The primary production of the Benguela region averages 735 gC·m^{-2}·year^{-1} (Brown *et al.* 1991), lower than the 1225 gC·m^{-2}·year^{-1} average of the Peruvian Current

(Walsh 1981). The average fish biomass for the Benguela region is estimated at 23 tonnes per square kilometre (Ware 1992).

Major changes have occurred in the system since the beginning of industrial exploitation and subsequent overfishing, including spatial changes in the distribution of small pelagic fish (Shannon *et al.* 2003; Shannon and Cury 2004), and increases in biomass of other species such as jellyfish (Flynn *et al.* 2012; Roux *et al.* 2013) and gobies(Utne-palm *et al.* 2010).

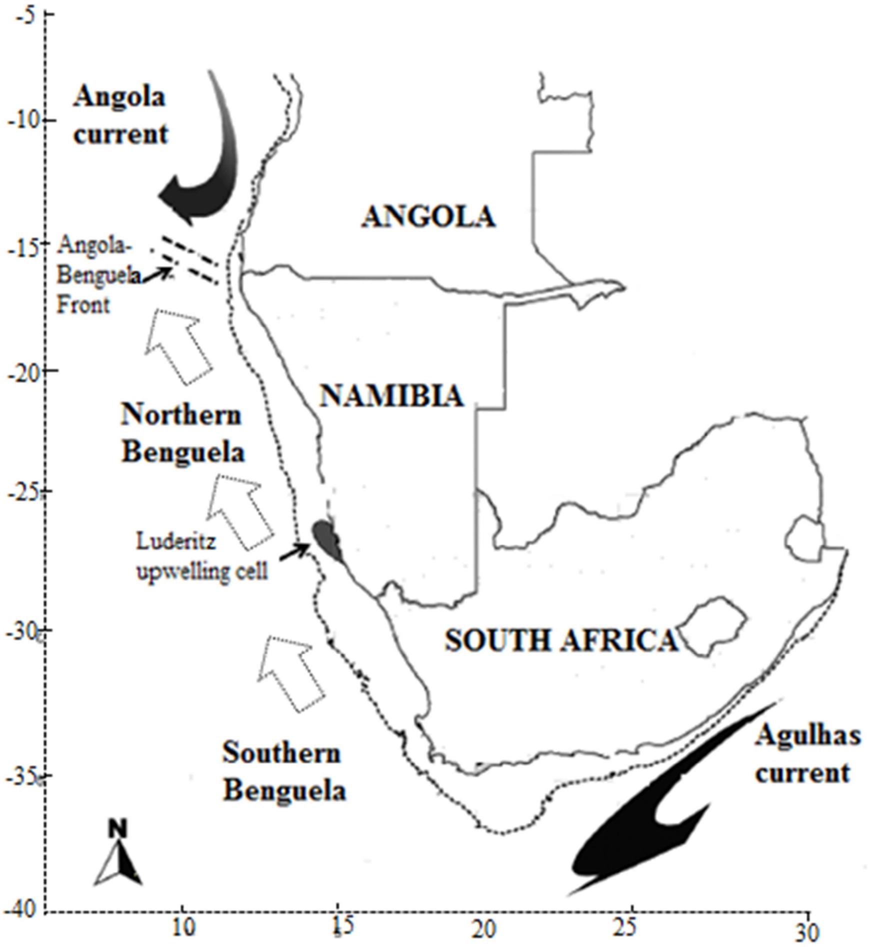

Figure 1.1: A general map of the Benguela Current ecosystem (modified from Roux *et al.* 2013).

1.2 Hake species off Namibia

The two hake species off Namibia (*Merluccius capensis* Castelnau 1851 and *M. paradoxus* Franca 1960) belong to the family Merlucciidae. Hake from the *Merluccius* genus occupied shallow epicontinental seas between Europe and Asia in the Paleogene period [(around 23 million years ago (Ma); (Svetovidov 1948)]. Gradual reduction in high-latitude ocean temperatures in the Oligocene [(around 34 Ma); (Savin *et al.* 1975)] forced the distribution of ancestral *Merluccius* southward (Inada 1981). The southern dispersals of *M. merluccius* 10-15 Ma and 2 Ma, respectively (Figure 2), have led to the present day populations of *M. paradoxus* and *M. capensis* in Namibian waters (Grant and Leslie 2001).

For decades, the two sympatric species (*M. capensis* and *M. paradoxus)* have been the main target of an important bottom trawl fishery off Namibia, with hake catches from 1973 to 1990 averaging 500 – 600 thousand tonnes annually (Westhuizen 2001). At the time of Namibian independence from South Africa, the newly formed government introduced management measures aimed at rebuilding Namibia's fish stocks, and since 1990 annual hake landings have ranged between 55,000 and 200,000 tonnes (Iyambo 2001).

Morphologically the two species differ in number of vertebrae, pigmentation on the gill rakers, and otolith and pectoral fin shape (Von Der Heyden *et al.* 2007 and references therein). The two species are distributed at overlapping depths, with *M. capensis* living in waters of ca. 50 to 350 m, and *M. paradoxus* from 250 to 600 m (Burmeister 2001). *Merluccius capensis* is more abundant than *M. paradoxus* in Namibian waters (Kainge *et al.* 2011)**.** These two species are found along the entire Namibian shelf, with smaller individuals of both species occurring in shallower waters (Burmeister 2001) and having diurnal variability in the sizes of the pelagic and demersal components (Iilende *et al.* 2001). *Merluccius capensis* is known to spawns in Namibian waters, while *M. paradoxus* appears to spawn only in South Africa (Kainge *et al.* 2007).

The two species are not distinguished in commercial hake catches (Gordoa *et al.* 2006), therefore both are managed as a single stock by the Namibian Ministry of Fishery and Marine Resources (Butterworth and Rademeyer 2005). A new method of splitting time-series of commercial hake catches between the two species has been developed and will allow for a species-specific hake assessment (Johnsen and Kathena 2012).

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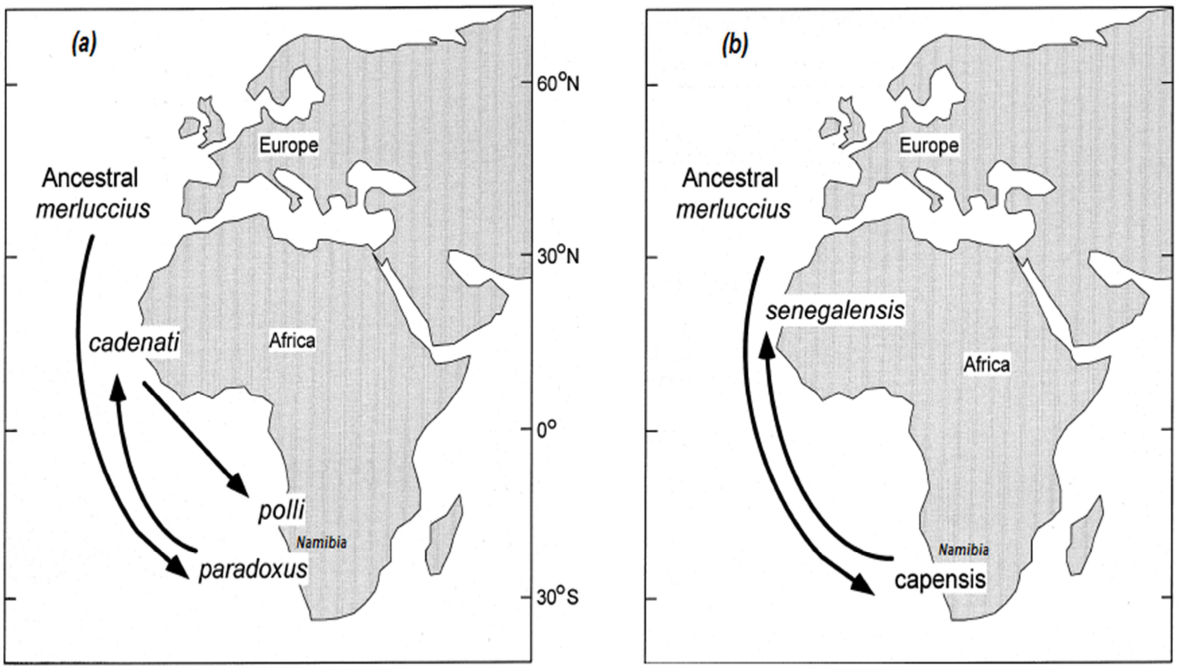

Figure 1.2: A geographic model of the origin of a) *Merluccius paradoxus* and b) *Merluccius capensis* (modified from Grant and Leslie 2001). The arrows represent the direction of the species dispersal.

1.3 Hake trophic relationships

The diets of the two hake species based on stomach content analyses were found to be similar and they are considered opportunistic predators of small fish and crustaceans (Roel and Macpherson 1988). Some of hake prey are krill, crustaceans, cephalopods, myctophids, horse mackerel, bearded goby, and other demersal and pelagic fish species (Macpherson and Roel, 1987; Traut, 1996). Larger hake of both species are considered cannibalistic (Payne *et al.* 1987), with *M. capensis* displaying a marked preference for small hake (Macpherson and Gordoa 1994). Human and several apex predators including seals (Mecenero *et al.* 2006) and sharks (Yano 1991; Ebert *et al.* 1992; Ebert 1994) feed on hake. Food-web models developed for the Benguela Current region have indicated that hake is the most important secondary consumer in the region (Heymans *et al.* 2004; Watermeyer *et al.* 2008). Inferences of the trophic relationships of *M. capensis* and *M. paradoxus* in the Benguela Current have mainly been based on stomach contents analyses (Macpherson and Roel 1987; Payne *et al.* 1987; Roel and Macpherson 1988; Macpherson and Gordoa 1994; Traut 1996)**,** but such data are limited temporally because they represent snapshots of recent feeding, and quantitatively because of biases resulting from variability in the digestion rates of different prey (Cortés 1997).

1.4 Assessment of the trophic relationships in the marine food web.

1.4.1 Introduction

In his seminal book " On the Origin of Species", Darwin pictured animal assemblages in nature as bound together by a web of complex linkages (Darwin 1859). Studies have indicated that different organisms are fixed in food webs within different ecosystems (Rosenzweig and Macarthur 1963; Schoener 1971; Paine 1988). Food webs have now long been described as consisting of complex species interactions through various food chains (Elton 1927, 1929; Hutchinson 1959; Paine 1963, 1969). Food chains are pathways of organic energy through trophic positions from the base to the top of a food web (Lindeman 1942; Post 2002a). Trophic positions of individual organisms and/or species depicts their location within food webs, with the maximum trophic position being the top level of the food web (Yodzis 1984; Williams *et al.* 2004). Trophic positions of species are difficult to determine due to the plasticity of their feeding (Pimm and Lawton 1977; Brodeur and Pearcy 1992), but conventional trophic level hierarchy starts from 1.0 for primary producers (basal species), and usually ends with third or fourth level consumers (Pimm and Kitching 1988; Williams *et al.* 2004). Energy transfer efficiency from one trophic level to the next is around 10% (Lindeman 1942)**,** although in marine ecosystems it can vary between 2 and 24% (Pauly and Christensen 1995; Gladyshev *et al.* 2011). Food webs often support large numbers of species interacting in complex networks of direct and indirect pathways (Martinez 1991; Polis 1991).

There are important differences between marine and other ecosystem (e.g. terrestrial and freshwater), with marine food webs having on average more trophic links per species (Cohen 1994; Link 2002a; Dunne *et al.* 2004; Warrant and Locket 2004). It is not clear whether these differences in food webs among ecosystem types arises from fundamentally different ecological processes, or from incidental consequences of evolutionary events (Webb 2012). Marine ecosystems are among the world's most productive and diverse systems (Solan *et al.* 2006). The base of most marine food webs consist of phytoplankton, with zooplankton being the major link between primary producers and higher trophic levels (Ramos *et al.* 2012). Many marine ecosystem have a very large numbers of species at the lower trophic levels, and a substantial numbers of predatory fishes that feed at the apex or near-apex levels, and a crucial intermediate trophic level of plankton-feeding pelagic fishes (Bakun 2006; Cury *et al.* 2011).

In any food web consisting of a network of organisms connected by their trophic interactions, there are differences in the relative strength and importance of these interactions (Paine and Schindler 2002). Assessing these trophic relationships is a daunting task, especially when considering the logistics of studying marine communities and upper-trophic level consumers, where year-round sampling of different components of the food web is highly problematic (Cortés 1999). Understanding of the trophic relationships among marine species is however crucial to managing our marine resources with higher certainty (Yodzis 1994; Mueter and Megrey 2006), and through a science-based ecosystem approach to fisheries management that considers multi-species trophic interactions (Roux and Shannon 2004; Garcia and Cochrane 2005; Cochrane *et al.* 2009; Paterson and Petersen 2010; Shannon *et al.* 2010; Paterson *et al.* 2010).

In addition to the conventional stomach content analyses, our understanding of trophic interactions in marine communities has been augmented using trophic interaction models (Berryman 1992; Giacomini *et al.* 2013) such as the Lotka-Volterra model (Lotka 1925) and ecosystem dynamic models such as Ecopath (Pauly *et al.* 2000). Over the last two decades, the use of stable isotope analysis and the analysis of fatty acid signatures in ecological research has increased (Grey *et al.* 2000; Arnould *et al.* 2005; Schmidt *et al.* 2006, 2007; Post *et al.* 2007; Koussoroplis et al. 2011; Parrish *et.al* 2012). The two methods provide temporally-integrated measures of trophic relationships among species and can detect trophic interactions not observed through stomach content analyses.

1.4.2 Stable isotopes

Stable isotope analysis has emerged as a method that has transformed our understanding of ecological systems (Haines 1976; West *et al.* 2006) by delineating trophic pathways and the relative trophic levels of consumers (Peterson and Fry 1987). Of the elements used (Table 1), stable isotopes of nitrogen and carbon have been the most widely used in ecological studies

(Peterson and Howard 1987; Behringer and Butler 2006; Stowasser *et al.* 2009; Choy *et al.* 2009; Iitembu *et al.* 2012)**.**

The stable isotope analysis approach is based on the principle that carbon and nitrogen isotope ratios in animal tissues closely reflect those of their assimilated prey, with a predictable enrichment of the heavier isotopes (^{13}C, ^{15}N) because of preferential metabolism of the lighter ^{12}C and ^{14}N isotopes (DeNiro and Epstein 1978, 1981).The isotopic ratios of these elements show predictable enrichment through food webs (Peterson and Fry 1987). During metabolism of nitrogen, the light isotope (^{14}N) is concentrated in nitrogenous excretory products while the heavy isotope(^{15}N) is retained in consumer tissues (Peterson 1999), with a trophic enrichment factor averaging ≈3.4 ‰ per trophic level (Minagawa and Wada 1984; Peterson and Fry 1987; Mccutchan *et al.* 2003). For carbon stable isotopes, different forms of primary producers fix the carbon (^{13}C and ^{12}C) isotopes at different rates; hence different producers can have disparate δ^{13}C signatures. These basal differences are then reflected in the consumers, as ^{13}C values in consumer tissues are enriched by ≈1‰ per trophic level (Vander Zanden *et al.* 1998; Post 2002b). The trophic enrichment factors (TEF) of both of nitrogen (^{15}N) and carbon (^{13}C) isotope, which are the differences in isotopic composition between an animal and its diet, can vary among species (Vanderklift and Ponsard 2003; Caut *et al.* 2009). Studies have identified large differences in TEF between taxon, species, tissues, diet quality environment and feeding rate (Hussey et al. 2010 and references therein).

One of the caveats for using stable isotopes in ecology is the time a tissue takes to equilibrate isotopically with that of an organism's diet, known as the isotopic turnover rate. The isotopic turnover rate in its simplest form is a function of tissue mass, metabolic fraction, and growth fraction (Hesslein *et al.* 1993), with the metabolic fraction in most fish being minimal, and almost all isotopic turnover occurring through somatic growth (Hesslein *et al.* 1993; MacAvoy *et al.* 2001; Miller 2006; Suring and Wing 2009). Smaller fish therefore express faster turnover rates than their larger counterparts (Jennings *et al.* 2008). The successful application of stable isotope analysis to animal ecology is therefore dependent on our knowledge of how animals incorporate the isotopic composition of their food into their tissues (Gannes *et al.* 1997). Trophic relationships can be difficult to resolve where the potential food sources are not isotopically distinct, or where a variety of potential food sources exist (Peterson 1999). Researchers have demonstrated that the combined use of stable isotopes and other methods such as fatty acid analysis can better reveal trophic relationships in situations where conventional methods alone previously provided insufficient data (Alfaro *et al.* 2006; El-Sabaawi *et al.* 2009; Stowasser *et al.* 2009)**.**

Table 1.1: Average abundances (on Earth) of stable isotopes important for understanding ecosystems (source: West *et al.* 2006).

Element	**Isotope**	**Average abundance (%)**
Hydrogen	^{1}H	99.985
	^{2}H	0.0015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{15}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulphur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{35}S	0.014

1.4.3 Fatty acids

Fatty acids are used as metabolic energy reserves and are accumulated in substantial amounts by many aquatic organisms (Pond 2012). Since Lovern (1935) suggested the conservative transfer of fatty acids (FAs) in aquatic food webs, FA analyses have been widely used in the trophic analysis of marine systems (Graeve *et al.* 1994; Alfaro *et al.* 2006; Rossi *et al.* 2006; Allan *et al.* 2010). The application of FAs as trophic markers has mostly been used to gain qualitative knowledge about the diets of consumers alone (Brett et al., 2006; Lança et al., 2011), to qualitatively investigate the dietary sources using a FA found in a predator and their prey (Prato et al., 2012; Shin et al., 2008)**,** and to quantitatively estimate diet from FA signatures of predator and prey (Iverson *et al.* 2004).

Fatty acids have been used as diet tracers in marine consumers because most consumers lack the desaturase enzymes needed to biosynthesize unsaturated FAs and therefore have to acquire these components from their food (Tocher and Ghioni 1999; Hastings *et al.* 2001; Nichols 2003)**.** Fatty acids are transferred through the food chain from phytoplankton that carry out elongation of short-chain compounds (Calder 2001; Nichols 2003)**,** and these longer chains are incorporated into consumer tissues without extensive modification (Dalsgaard *et al.* 2003; Haubert *et al.* 2011). Neutral lipid classes are used as energy stores by organisms, while polar lipid classes are important constituents of cell membranes (Dalsgaard et al., 2003 and references therein). As membrane components, the fatty acids in polar lipids may be elongated and desaturated by organisms(Caramujo *et al.* 2008), but can provide long-term dietary information about a consumer (Koussoroplis *et al.* 2010). Fatty acids in neutral lipids can be deposited into tissues relatively unmodified during trophic transfer (Caramujo *et al.* 2008) and generally resemble the consumer’s food intake more closely than polar lipids (Jezyk and Penicnak 1966; Parrish *et al.* 1995). The caveat in using FAs in food web studies is that consumer FA signatures will never directly match dietary FA composition because of *de novo* synthesis of FAs, FA modifications, and selective catabolism and/or storage of FAs (Williams and Buck 2010). However, as more controlled feeding studies are completed, researchers will gradually improve upon the amount of accurate dietary information that can be extracted from fatty acid profiles of food and consumers. In spite of the above-mentioned caveats, qualitative and quantitative dietary inferences on the influence of diet on the FA composition of various tissues can still be made (Budge *et al.* 2002, 2006; Richoux 2005, 2010; Connan *et al.* 2010; Beckmann *et al.* 2013).

1.5 Thesis overview

Several studies based on inferences from stomach content analyses (Macpherson and Roel 1987; Payne *et al.* 1987; Roel and Macpherson 1988; Macpherson and Gordoa 1994) indicated that hake are important secondary consumers in the Benguela Current region; however, the trophic relationships of hake (*M. capensis* and *M. paradoxus*) in Namibian waters are not well defined. The main objective of this thesis was to increase our knowledge of the two species' trophic relationships using time-integrating trophic tracers by:

1. investigating the relative ontogenetic trophic shifts in hake using $\delta^{15}N$, overlap of diet using $\delta^{13}C$, and the relative trophic levels of their dominant prey (Chapter 3),
2. comparing the relative contributions of different potential prey to the diets of the two hake species using a Bayesian isotope mixing model (Chapter 4),
3. measuring fatty acid signatures in hake and relating them to potential prey (Chapter 5), and
4. examining some aspects of the trophic relationships of the two hake species relative to demersal sharks off Namibia using $\delta^{15}N$ and $\delta^{13}C$ of their tissues (Chapter 6).

Results herein will help the Namibian Ministry of Fisheries and Marine Resources to implement a science-based ecosystem approach to fisheries management (Cochrane et al. 2009) and researchers to construct accurate trophic models of the northern Benguela (Namibia) Current ecosystem.

1.6 Publication status and co-authorship statements

Chapter 3 is published as: Iitembu J.A., Miller T.W., Ohmori K., Kanime A. and Wells S. (2012) Comparison of ontogenetic trophic shift in two hake species, *Merluccius capensis* and *Merluccius paradoxus*, from the Northern Benguela Current ecosystem (Namibia) using stable isotope analysis. *Fisheries Oceanography* 21, 215–225. I developed the concepts in the paper, designed the sampling protocols, carried out laboratory analyses, conducted statistically analyses and wrote the manuscript. Dr Miller assisted with the manuscript writing and statistical analyses, Dr Ohmori assisted with editing the manuscript, Mr Kanime and Mr Wells assisted with laboratory analyses and samples collection. The paper (Chapter 3) is reproduced with permission (Appendix 2).

Chapter 4, 5 and 6 are finished manuscripts. I developed the overall concepts, designed the sampling protocols, directed the sampling team, carried out laboratory and statistical analyses, and wrote the manuscripts. Drs Richoux and Miller provided supervisory directions, ideas and editorial suggestions.

CHAPTER 2: MATERIALS AND METHODS

2.1 Field sampling and sample preparation

Samples of hake, sharks and their potential prey were collected in the northern Benguela upwelling system (Namibia) (Fig 2.1). The collection was done during hake (*M. capensis* and *M. paradoxus)* biomass surveys (10th January -15th February 2008, 10th January-19th February 2009, 12th January –24th February 2011, 11th January –25th February 2012) on board MV *Blue Sea I* and a monkfish (*Lophius vomerinus*) biomass survey (16th -27th December 2011) on board *RV Welwitchia***.** These surveys were mainly done for estimation of biomass and size composition of the hake and monkfish stock off Namibia, so sampling followed survey pre-determined stations and was confined within the borders of Angola (latitude 17°12'S) and South Africa (29°30'S) at a depth range of 90 – 700 m (Fig 2.1).

Sampling during hake biomass surveys was done using a Gisund Super two-panel bottom trawl (head length 31 m, footrope 47 m, and the vertical net opening 4.5–5.5 m), while sampling during the monk biomass surveys was performed using a commercial type Albatross monkfish bottom trawl (head length 50.3 m, footrope 63.9 m and the vertical net opening 1.2-1.3 m) rigged with tickler chains along the footrope. Trawling speed during both surveys was on average 3.0 knots, and trawling time was 30 minutes except when trawling was interrupted due to a large catch, or a rough/rocky or muddy bottom.

Sampling for all fish was opportunistic, with the general goal of obtaining a wide size distribution of each species to examine their trophic relationships. At each station where sampling was done (Fig 2.1), individual fish (ranging from 1 to 10, if present in the catch) were chosen from the largest, middle and smallest size groups. After identification, each fish was measured (total length). A small section of white muscle was removed from the anterior-dorsal region of each fish (hake, potential prey and sharks) selected for deriving stable isotope data (Table 2.1). Skin was removed from the muscle, and the muscle was immediately wrapped in aluminium foil and frozen at -20°C on the ship. Samples were taken to the National Marine Information and Research Centre (NatMRIC, Swakopmund, Namibia) and Sam Nujoma Marine and Coastal resources Research Center (SANUMARC, Henties bay, Namibia) for laboratory preparations. Tissue samples were dried for 48 hours at 60°C, ground to a fine powder using a mortar and pestle, and placed in polyethylene vials for storage. The remaining whole fish were stored in Ziploc bags and frozen at -20 °C so that additional fish tissues could be later dissected from the same specimens for fatty acid sample processing (Table 2.1). The additional muscle tissue samples were removed from each frozen fish as described above, stored at -80°C for 24 hours and then lyophilized at -60°C for 24 hours.

Some prey samples were collected from hake stomach contents (see chapter 3, Table 3.1 for more details). Nineteen stomach samples from *M. capensis* and 15 stomachs of *M. paradoxus* were analysed from the August 2009 port samples. Stomachs were first removed from the fish and assessed for digestive condition. Stomachs with fresh prey items were retained and placed in a plastic bag and immediately frozen at -20°C for further identification, processing and analysis. Large fish prey were identified to the lowest taxonomic level, rinsed gently with distilled water to remove gut mucous, and the dorsal anterior muscle was removed for isotope analysis. For zooplankton such as euphausiids and shrimp, individuals were identified, rinsed with distilled water and whole bodies saved for isotope analysis. Fresh stomach contents for stable isotope analysis have been used in ecological studies (Sholto-Douglas *et al.* 1991; Tanaka *et al.* 2008).

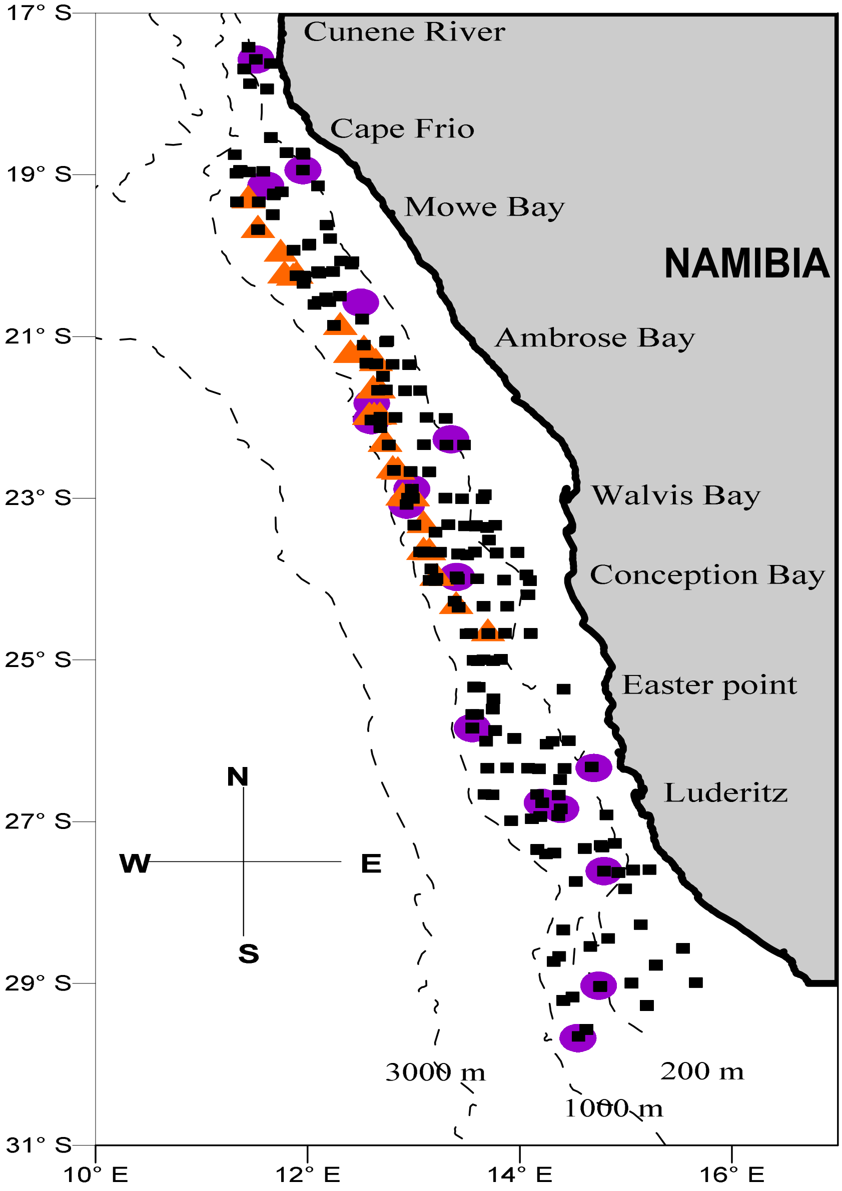

Figure 2.1: A layout of geographical positions where samples for hake (black squares), prey (purple circles) and shark (orange triangles) were collected off Namibia. Depth contours represent 200, 1000 and 3000 m.

Table 2.1: Sampling information of specimens taxa, total number of samples (*n*), number of samples per sampling period and their use in this study (SI= stable isotope and FA= fatty acid).

		Number of samples per collection period						
	n	Jan-Feb 2008	Jan-Feb 2009	August 2009	Jan-Feb 2011	Dec 2011	Jan – Feb 2012	Used for
Crustaceans								
Copepoda	3	-	-	3	-	-	-	SI
Euphausiids (*Euphausi*a sp).	34	-	-	18	-	-	16	SI
Shrimp (Penaeidae family)	3	-	-	3	-	-	-	SI
Shrimp (Pisiphaeidae family)	1	-	-	1	-	-	-	SI
Stripped red shrimp (*Aristeus varidens)*	19	-	-	-	-	-	19	SI
African mud shrimp *(Solenocera africana)*	10	-	-	-	-	-	10	SI
Golden shrimp *(Plesionika martia)*	19	-	-	-	-	-	19	SI
Woodward shrimp *(Funchalia woodwardi)*	16	-	-	-	-	-	16	SI
Cephalopods								
Cock-eyed squid (Histioteuthidae family)	1	-	-	1	-	-	-	SI
Bobtailed squid (Sepiolidae family)	3	-	-	3	-	-	-	SI
Flying squid *(Todarodes sagittatus)*	2	-	-	-	-	2	-	SI+FA
Lesser flying squid *(Todaropsis eblanae)*	6	-	-	-	-	6	-	SI

Table 2.1 continued

Teleosts								
Shortnose greeneye (*Chlorophthalmus agassizi*)	15	-	-	1	-	14	-	SI+FA
Cape dragonet *(Paracallionymus costatus)*	5	-	-	-	-	-	5	SI+FA
Light fish *(Phosichthys argenteus)*	11	-	-	-	-	11	-	SI
Cape bonnetmouth *(Emmelichthys nitidus nitidus*	4	-	-	-	-	-	4	SI
Pelagic goby *(Sufflogobius bibartus)*	5	-	-	-	-	-	5	SI+FA
Pencil cardinal *(Epigonus denticulatus)*	5	-	-	-	-	5	-	SI+FA
Blackbelly rosefish (*Helicolenus dactylopterus*)	27	-	-	3	8	16	-	SI+FA
Grenadiers (Macrouridae family)	6	-	-	6	-	-	-	SI
Grenadier *(Caelorinchus simorhynchus)*	7	-	-	-	-	7	-	SI
Smalltooth grenadier (*Nezumia micronychodon*)	33	-	-	10	12	11	-	SI+FA
Myctophids (Myctophidae family)	20	-	-	4	-	4	12	SI
Lantern belly (*Synagrops microlepsis*)	30	-	-	1	-	26	3	SI+FA
Cape gurnard *(Chelidonichthys capensis)*	6	-	-	-	-	6	-	SI
Horse mackerel (*Trachurus capensis*)	12	-	11	1	-	-	-	SI
Cape anchovy (*Engraulis capensis*)	119	-	105	-	-	-	14	SI+FA

Table 2.1 continued

Sardine (*Sardinops sagax*)	23	-	23	-	-	-	-	SI
Round herring (*Etrumeus whiteheadi*)	10	-	2	-	-	8	-	SI
Shallow water Cape hake (*Merluccius capensis*)	187	128	-	-	25	34	-	SI +FA
Deep water Cape hake (*Merluccius paradoxus*)	171	120	-	-	15	36	-	SI +FA
Arrow head dogfish *(Deania profundorum)*	15	-	-	-	15	-	-	SI
Bird-beak dogfish *(Deania calcea)*	9	-	-	-	9	-	-	SI
Leaf scale gulper shark *(Centrophorus squamosus)*	18	-	-	-	18	-	-	SI

2.2 Laboratory Analysis

2.2.1 Stable isotope Analysis

Isotope samples collected in 2008 and 2009 (used in chapter 3) were analysed at the Center for Marine Environmental Studies (CMES), Ehime University (Japan) using a Carlo Erba Elemental Analyzer 2500 coupled to a Finnigan MAT Delta Plus stable isotope ratio mass spectrometer via a ConFlo-III continuous flow interface. The standard applied at CMES was L-histidine calibrated to atmospheric N2 for δ^{15}N and Vienna Pee Dee Belemnite for δ^{13}C. Measurement error (SD) at CMES for both carbon and nitrogen was ± 0.3. Samples collected during 2011 and 2012 (used in chapter 4 and 6) were analysed at IsoEnvironmental CC Laboratory at Rhodes University (Grahamstown, South Africa) using a Europa Scientific Elemental Analyzer coupled to a 20-20 Isotope Ratio Mass Spectrometer (IRMS). Beet sugar, ammonium sulfate and casein were used as in-house standards at IsoEnvironmental CC Laboratory. These in-house standards were calibrated against International Atomic Energy Agency (IAEA) standards CH-6 and N-1. Measurements error (SD) at IsoEnvironmental CC Laboratory was of ±0.06 ‰ for δ13C and ±0.12 ‰ for δ15N.

The ^{12}C/^{13}C and ^{14}N/^{15}N isotope measurements were expressed in the delta notation relative to the levels of ^{13}C in Pee Dee Belemnite and ^{15}N in atmospheric nitrogen (N_2), according to the following equation: $\delta X = \{(R_{sample}/R_{standard}) - 1\} \times 1000$, where X is ^{13}C or ^{15}N and R is the ratio of the heavy to light isotope for the sample (R_{sample}) and standard ($R_{standard}$) in units of parts per thousand (‰).

Because carbon isotope ratios can be altered by changes in lipids irrespective of the carbon source (McConnaughey and McRoy 1979)**,** measurements were mathematically corrected for lipid variations using $\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N$, a normalization equation from Post *et al.* 2007b). δ^{13}C untreated is the δ^{13}C of non-lipid extracted tissue. The δ^{13}C of samples having C:N ratios <3.5 do not benefit from lipid normalization (Post *et al.* 2007), therefore only samples expressing C:N ratios >3.5 were lipid normalized. Lipid extraction was not performed as it can alter δ^{15}N values, requiring separate analyses of δ^{13}C and δ^{15}N analysis (Sweeting *et al.* 2006). The mathematical lipid correction method has worked well for lipid correction of δ^{13}C in deep-sea fishes (Hoffman and Sutton 2010).

To calculate the relative trophic level (TL) of different species, the following equation was used: Trophic level = $[(\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta\delta^{15}N] + 2.0$, where $\delta^{15}N_{consumer}$ is the signature of the consumer, $\delta^{15}N_{base}$ is the baseline value of the food web, $\Delta\delta^{15}N$ is the trophic enrichment factor, and the value 2.0 indicates the trophic level of the organism used to establish the $\delta^{15}N_{base}$. The trophic enrichment factor was set at 3.4‰ per trophic level (Minagawa and Wada 1984; Post 2002b). $\delta^{15}N$ of mytilid bivalves (TL=2.0) were used as $\delta^{15}N_{base}$ because bivalves are primary consumers that have protracted isotopic turnover rates that can integrate seasonal variability (Lorrain *et al.* 2002; Fukumori *et al.* 2008).

In chapter 3, a mean $\delta^{15}N$ of 9.2 ‰ from two mytilid bivalve species (*Choromytilus meridionalis* and *Mytilis galloprovincialis*) was used as $\delta^{15}N_{base.}$ The bivalves were collected around the same time as Chapter 3 samples by Kohler et al (2011) off Lüderitz (Namibia). In chapters 4 and 6, a mean of 9.8 ‰ from bivalve species (*Choromytilus meridionalis*), collected from Lüderitz (south coast, n=6), Swakopmund (central coast, n=6) and Henties bay (northern coast, n=6), was used as $\delta^{15}N_{base}$. The bivalves were collected at the same times as samples for chapters 4 and 6. The use of bivalves as the $\delta^{15}N_{base}$ was based on their isotopic value proximity to zooplankton (size 200–500 lm) in this study and that of Sholto-Douglas et al. (1991) west of South Africa, which in most cases were identical or very similar. I assumed no significant near-shore ⁄ offshore differences in $\delta^{15}N$ based on Holmes et al.'s (1998) observation of little change in $\delta^{13}C$ and $\delta^{15}N$ values of surface sediments up to 200 km off- shore; this matches known cross-shelf and offshore transport of upwelled water by strong and persistent upwelling within the region (Duncombe Rae 2005).

2.2.2 Fatty acid analysis

All fatty acid analyses were completed in the Fatty Acid Facility at Rhodes University (Grahamstown, South Africa). Lyophilized samples were individually ground with a mortar and pestle into a fine powder and a weighed mass (50-100 mg) was placed in glass tubes, after which chloroform with 0.01% butylated hydroxytoluene was added and the tube flushed with nitrogen (N_2). The tubes were sealed with teflon tape for storage at -20 °C. All materials used in the preparation of samples were cleaned with three rinses of methanol (MeOH) followed by three rinses of chloroform ($CHCl_3$), while glass tubes and pipettes were heated to 450 °C for four hours in a muffle furnace to remove organic contaminants.

Total lipid extraction was done using a modified Folch procedure (Folch *et al.* 1957), where samples were extracted in a 2:1 (v/v) chloroform/methanol solution and then the volume of solvents adjusted to an 8:4:3 ratio (v/v/v) of chloroform/methanol/water**.** Total lipids of hake tissues were fractionated into neutral (NL) and polar lipids (PL) using column chromatography on silica gel. A small amount of pre-combusted glass wool was placed in the tapered end of a Pasteur pipette, packed with approximately 0.8 g of silica gel, activated by heating at 100°C for 1 h and cooled for 30 minutes. The lipid extract in chloroform was placed at the top of the column, and NLs were recovered with approximately 6 mL of 98:1:0.5 chloroform/methanol/formic acid and then 3 ml of $CHCl_3$ were passed through the column to return it to neutral polarity. Polar lipids were eluted with 6 ml of MeOH following the method by Budge and Parrish (2003) .

Fatty acid methyl esters (FAMEs) of neutral and total lipids were prepared using the method of Budge *et al*. (2006), after 0.3 ml of a standard **(** 7-10 mg of 19:0 in 10 mL of $CHCl_3$) was added to each sample to allow for FAME quantification. Gas chromatography (GC) analyses of FAMEs were analysed using an Agilent 7890A GC fitted with a flame ionization detector and a Zebron-Waxplus 320 capillary column. The oven temperature was set at 70 ºC and held for 1 min, then increased at a rate of 40 °C/min to 170 °C and held for 3 min, and increased to 250 °C at 2.5 ºC/min and held for 4.5 min, for a total run time of 40 min per sample. Helium was used as the carrier gas at a flow rate of 1.6641ml /min, and the injector and detector were maintained at 250 °C and 300 °C, respectively. Peaks of fatty acids were integrated using Agilent ChemStation and confirmed on a subset of samples using an Agilent 7000A GC/MS-QQQ coupled with the NIST 08 MS library. Each fatty acid was measured as a proportion of the total fatty acids (%) and as fatty acid weight per dry mass using the internal standard peak areas. Fatty acids were named according to the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) for carbon chain length, number of double bonds and position of the double bond closest to the omega carbon.

2.3 Statistical Analysis

Details on statistical procedures are included in sections 3.2.3, 4.2.3, 5.2.3, and 6.2.3.

CHAPTER 3: ONTOGENIC CHANGES IN THE TROPHIC RELATIONSHIPS OF *MERLUCCIUS CAPENSIS* AND *M. PARADOXUS* USING A STABLE ISOTOPE APPROACH[1]

3.1 Introduction

Knowledge of life history characteristics of fish species and interactions of prey and predatory species is increasingly important as the world moves towards ecosystem-scale approaches to fisheries management. One of the principal challenges in aquatic ecology is determining how fish are distributed among available habitats, and how these distributions change with growth (Persson and De Roos 2003)**.** Understanding the growth-based changes in feeding behaviour is essential in understanding complex food web relationships (Werner and Gilliam 1984; Gilljam *et al.* 2011), as body size is an important feature that determines potential predators and prey (Jennings *et al.* 2001; De Roos and Persson 2002).

Two sympatric species of hake are distributed at overlapping depths, with *Merluccius capensis* living in waters of ca. 50–350 m, and *M. paradoxus* in deeper waters from 250–600 m (Burmeister 2001). Studies using stomach contents indicated that both species are important opportunistic predators within the Benguela Current ecosystem (Roel and Macpherson 1988; Traut 1996). Interpretations of stomach content data are, however, limited (see Chapter 1) as these data are incapable of providing precise and accurate parameters for multi-species models (Punt *et al.* 1992).

Despite ecological significance of hake (Heymans *et al.* 2004; Watermeyer *et al.* 2008), their trophic relationships and relative trophic positions in the food web are not well understood. In many population, organisms undergo ontogenetic niche shifts with respect to resource and/or habitat use (Jensen *et al.* 2012). Previous studies using stomach content data indicate that both hake species become increasingly piscivorous through their development (Roel and Macpherson 1988; Punt *et al.* 1992).

Given the strong morphological similarities of the two species of hake and their known overlap in depth distribution, there is a need to determine their spatial and ontogenetic trophic

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[1] Published as Iitembu J.A., Miller T.W., Ohmori K., Kanime A. and Wells S. (2012) Comparison of ontogenetic trophic shift in two hake species, *Merluccius capensis* and *Merluccius paradoxus*, from the Northern Benguela Current ecosystem (Namibia) using stable isotope analysis. Fisheries Oceanography 21, 215–225.

overlap, which can provide essential information for dynamic ecosystem models and fisheries management. Stable isotope analysis is useful because of its time- and space-integrated representation of diets (Bearhop *et al.* 2004; Layman *et al.* 2007). This technique can allow identification of the nature of dietary shifts through ontogeny, specifically concerning the shifts in niche width, niche position and niche overlap (Hammerschlag-Peyer *et al.* 2011). In this study, carbon and nitrogen stable isotope analysis was applied to examine the trophic dynamics of *M. capensis* and *M. paradoxus* with respect to: i) their ontogeny, ii) spatial variations, and iii) potential prey. It was hypothesized that there are differences in the ontogenetic trophic shifts of the two species and isotopic spatial variations (depth and latitude) because of differences in their distributions. This chapter represents the first comprehensive isotope analysis of two sympatric hake species in the Benguela Current region.

3.2 Methods and materials

3.2.1 Sample collection

All hake samples were collected during a hake biomass survey (January- February 2008) as described in Chapter 2, section 2.1. The prey were collected from stomach contents and field sampling as described in Chapter 2, section 2.1.

3.2.2 Laboratory analysis

All samples were measured for $\delta^{13}C$ and $\delta^{15}N$ at the Center for Marine Environmental Studies, Ehime University (Japan) as described in Chapter 2, section 2.2.1.

3.2.3 Statistical Analysis

Analysis of variance (ANOVA) and linear regression methods were applied to examine the relationship of stable isotope ($\delta^{15}N$ and $\delta^{13}C$) values to size (length), depth, latitude, and in some instances interactions between parameters. Prior to analyses, data were examined for normality and homogeneity of variance using Shapiro-Wilk’s test and Levene’s test, respectively. Violations of either were addressed using log transformation of the data.

Size-specific differences between *M. capensis* and *M. paradoxus* were explored using ANCOVA, with isotopes ($\delta^{13}C$ and $\delta^{15}N$) as the dependent variables, species (*M. capensis* and *M. paradoxus*) as the dummy variable, size, latitude and depth as covariates, and the interaction term of length×species. The interaction term determines whether there is a significant difference between the slopes of the isotopic values in the two species, which determines any size-specific differences in trophic behaviour. Species differences in $\delta^{13}C$ and $\delta^{15}N$ by length classes (total length) of 20-29, 30-39, 40-49, 50-59, and 60-69 cm (following Roel and Macpherson 1988) were examined using a t-test comparison ($\alpha=0.05$). Low sample sizes of hake ≥70 cm were deemed unsuitable for robust statistical analysis. From all size-specific comparisons, variances in $\delta^{15}N$ between *M. capensis* and *M. paradoxus* were not equal (Levene's test, $P = 0.03$). All statistical tests were performed using R (R Core Team (2009), Vienna, Austria).

3.3 Results

Comparisons of $\delta^{15}N$ between *M. capensis* and *M. paradoxus,* after accounting for covariates of length and the interaction term (length×species), showed a moderately insignificant species difference in $\delta^{15}N$ (ANCOVA, $p = 0.056$, $F(1, 224) = 3.6$), with both length (ANCOVA, $p < 0.001$, $F(1, 224) = 81.6$) and the interaction term length×species (ANCOVA, $p < 0.001$, $F(1, 224) = 12.7$) being highly significant (full model, $p < 0.001$, $R^2 = 0.33$). From the regression of $\delta^{15}N$ and length between the two species, *M. paradoxus* displayed a stronger relationship ($p <0.001$, $R^2 = 0.50$) than *M. capensis* ($p < 0.001$, $R^2 = 0.10$). For both species, length was the only significant variable in explaining $\delta^{15}N$ (Fig. 3.1), even after considering variables of depth, latitude, and interaction terms. In particular, *M. capensis* did show a significant correlation between total length and depth ($p < 0.001$, $R^2 = 0.26$), but when length and depth plus their interaction were applied to explain $\delta^{15}N$, depth and the interaction term of depth × length were found non-significant ($p = 0.11$ and 0.09, respectively; full model $R^2 = 0.51$). Size-specific comparisons between the two species showed no statistical differences in $\delta^{15}N$ between size classes from 20-39 cm ($p = 0.3$, mean difference $\delta^{15}N <0.1‰$), but sizes 40-49 cm ($p = 0.05$, mean difference $\delta^{15}N = 0.3‰$) and larger ($p \leq 0.005$) were significantly different, with *M. paradoxus* on average higher than *M. capensis* (Fig.3.1).

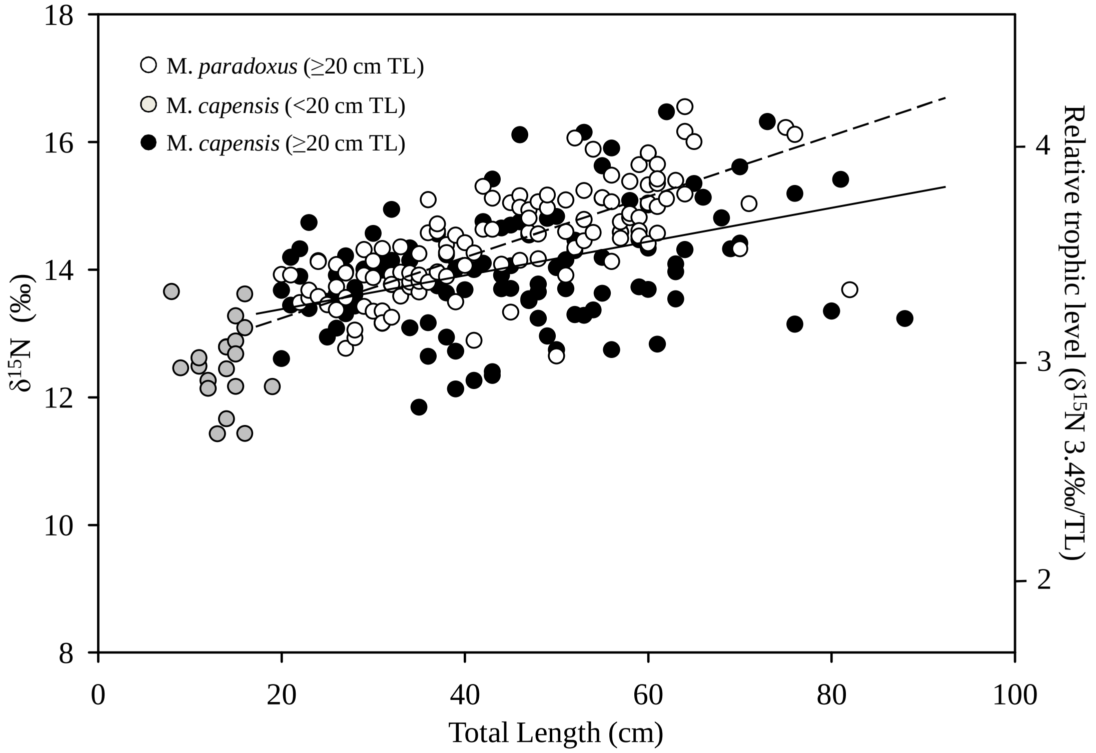

Figure 3.1: Stable isotope plot of δ^{15}N and Total Length (cm) by relative trophic level (TL) of individual *Merluccius capensis* and *Merluccius paradoxus*. Relative trophic level was approximated from mean δ^{15}N values of zooplankton primary consumers (copepods, shrimp and euphausiids), with subsequent increasing TLs every 3.4‰ in δ^{15}N. Dashed line denotes least squares for *M. paradoxus* for fishes ≥20 cm total length (β=0.04, t (1, 115) =72.1, F (1,105) = 148.6, R_2=0.56, p<0.001). Full line denotes least squares for *M. capensis* ≥20 cm total length (β=0.02, t (1, 105) = 4.4, F (1,105) = 19.6, R_2 = 0.16, p <0.01).

For δ^{13}C, a significant positive relationship with size was only observed in *M. capensis* (p < 0.001, R^2 = 0.10). Comparison of size-specific δ^{13}C values between *M. capensis* and *M. paradoxus* showed no significant differences in the smallest size class of 20-29 cm (p = 0.4, mean difference in δ^{13}C = 0.1‰), but significant differences in size categories ≥30-39 cm were observed (p = 0.02, mean difference in δ^{13}C = 0.3‰, Fig. 3.2). For both species, depth and latitude were not influential explanatory variables (*P* > 0.10).

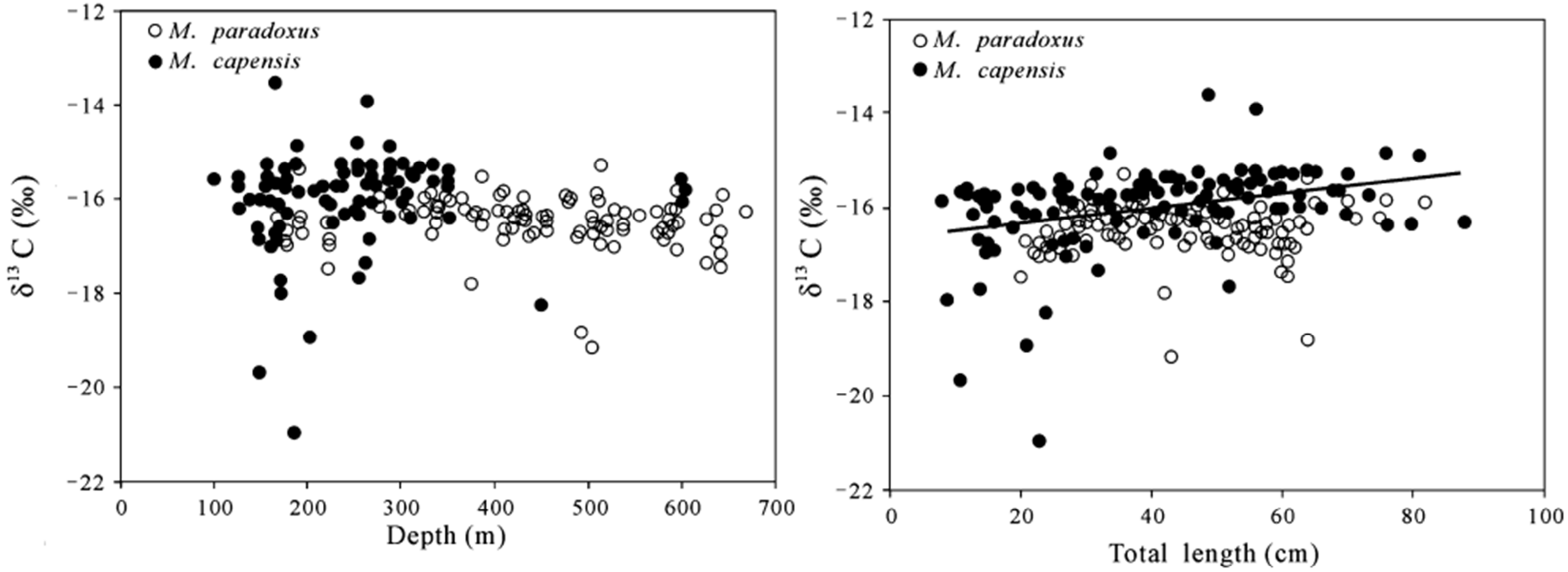

Figure 3.2: Plots of *M. capensis* and *M. paradoxus* δ^{13}C values versus water column depth of collection (top) and total length (bottom). Depth was not significant for both species (p > 0.20, both *M. paradoxus* and *M. capensis*). Total length was significantly influential in *M. capensis* [β = 0.02, t (1, 107) = 70.9, F (1, 107) = 11.4, R_2 = 0.10, p = 0.001], denoted as the least squares line.

Analysis of hake prey showed most fishes displaying enriched $\delta^{15}N$ values and relative trophic levels compared to squid and crustaceans (Fig. 3.3, Table 3.1). Sardine had the most depleted mean $\delta^{15}N$ values of all fishes (mean ± SD = 9.2‰ ±0.7), comparable to copepods and euphausiids (mean $\delta^{15}N$ = 9.6‰ ±0.2 and 9.7‰ ±1.2, respectively). Using a fractionation value of 3.4‰ per trophic level from the baseline of mytilid bivalves from Kohler *et al.* 2011 (mean $\delta^{15}N$ = 9.2‰), all hake (both *M. paradoxus* and *M. capensis*) >20 cm total length had $\delta^{15}N$ values above zooplankton and nekton prey, and had TLs of 3.0 and 3.8 (Table 3.1, Fig. 3.3).

From $\delta^{13}C$ of all zooplankton and nekton, enriched (least negative) values of >-16.0‰ were expressed in larger *M. paradoxus* (>30 cm total length), small tooth grenadier and other grenadiers (Macrouridae), sardine (*Sardinops sagax*) and anchovy (*Engraulis capensis).* Intermediate values between -16.0 and -17.0‰ were observed in *M. paradoxus*, large horse mackerel (*Trachurus capensis*), round herring (*Etrumeus whiteheadi*), cockeyed squid (Histioteuthidae), blackbelly rosefish (*Helicolenus dactyloptera*), shortnose greeneye (*Chlorophthamus agassizi*), pasiphaeid shrimp and euphausiids (*Euphausia* sp.). The most depleted (most negative) values <-18.0‰ were in copepods, *Synagrops* (Perciformes), myctophids, and penaeid shrimp.

Table 3.1: **S**table isotope measurements [‰ (standard deviation)] of zooplankton and nekton taxa with their average total length [L (cm)], number of samples (N), sample source [(stomach (s) or field (f) collection)], carbon: nitrogen ratio (C: N) and trophic level (TL). δ^{13}C' represents the lipid-normalized values and code represents the Fig. 3.2 codes.

Taxa $^{\text{sample source}}$	**code**	**L**	**N**	**δ^{15}N**	**C:N**	**δ^{13}C**	**δ^{13}C'**	**TL**
Zooplankton (length = total length mm)								
Copepoda$^{(f)}$	cope	<1.0	3	9.6(0.2)	4.2(0.2)	-20.4(1.2)	-19.6 (0.1)	2.1(0.1)
euphausiids – *Euphausia* sp.$^{(s)}$	euph	15.0	18	9.7(0.3)	4.0(0.4)	-17.5 (0.5)	-16.8(0.6)	2.1(0.3)
Penaeidae – shrimp$^{(s)}$	pena	-	3	9.2 (0.7)	5.1(0.1)	-18.5 (0.2)	-16.8(0.2)	2.0(0.2)
Pasiphaeidae – shrimp$^{(s)}$	pasi	-	1	11.5	3.6	-17.1	-16.9	2.7
Cephalopoda								
Histioteuthidae - cockeyed squid$^{(s)}$	histio	-	1	12.4	3.7	-16.0	-15.7	2.9
Sepiolidae - bobtailed squid$^{(s)}$	sepi	-	3	11.2(0.2)	3.6(<0.1)	-16.4(<0.1)	-16.2	2.6
Fish (length = total length cm)								
Shortnose greeneye (*Chlorophthalmus agassizi*) $^{(s)}$		12.0	1	12.2	3.2	-17.2		2.9
Blackbelly rosefish (*Helicolenus dactylopterus*) $^{(s)}$		14.0	3	10.9(0.4)	3.8(0.2)	-16.8 (0.4)	-16.3(0.3)	2.5 (0.1)
Macrouridae – grenadiers$^{(s)}$	macr	12.0	6	12.2(0.3)	3.3 (0.1)	-14.7 (0.4)		2.8 (0.1)
Smalltooth grenadier (*Nezumia micronychodon*) $^{(s)}$		19.2	10	12.7(1.4)	3.2(0.1)	-15.2(0.4)		3.0(0.4)
Myctophidae$^{(s)}$	myct	5.5	4	11.7(1.0)	5.7(1.9)	-18.9(1.5)	-1.66(0.7)	2.7(0.3)
Acropomatidae (*Synagrops* sp.)$^{(s)}$		-	1	14.6	7.9	-19.5	-15.0	3.6
Horse mackerel (*Trachurus capensis*), small$^{(s)}$	tcap (S)	18.7	1	11.5	6.2	-19.6	-16.9	2.8
Horse mackerel (*T. capensis*), large$^{(f)}$	tcap (L)	40.7	11	13.6(0.4)	3.4(0.2)	-16.5(0.4)		3.3(0.1)
Cape anchovy (*Engraulis capensis*) $^{(f)}$	anch	9.3	105	12.1(1.1)	3.7(0.3)	-16.2(0.6)	-15.8(0.5)	2.7(0.3)

Table 3.1 continued

Sardine (*Sardinops sagax*) (f)	sard	18.3	23	9.2(0.7)	3.4(0.2)	-15.9(0.7)	2.0
Round herring (*Etrumeus whiteheadi*) (f)	redeye	11.5	2	12.8	3.5	-16.7	3.1
Shallow water Cape hake (*Merluccius capensis*) (f)	MC$_{8-19}$	13.6	19	12.5(0.6)	3.2(0.1)	-16.5(1.0)	3.0(0.2)
	MC$_{20-29}$	24.8	22	13.7(0.5)	3.2(<0.1)	-16.5(1.3)	3.3(0.1)
	MC$_{30-39}$	34.9	22	13.6(0.8)	3.1(0.1)	-16.0(0.6	3.3(0.2)
	MC$_{40-49}$	44.9	24	13.9(0.2)	3.2(0.1)	-15.7(0.6)	3.4(0.3)
	MC$_{50-59}$	53.9	20	14.2(1.0)	3.2(0.1)	-15.8(0.7)	3.5(0.3)
	MC$_{60-69}$	63.9	13	14.4(0.9)	3.1(<0.1)	-15.7(0.4)	3.5(0.3)
	MC$_{70+}$	76.8	8	14.6(1.2)	3.2(<0.1)	-15.8(0.7)	3.6(0.4)
Deep-water Cape hake (*M. paradoxus*) (f)	MP$_{20-29}$	25.5	19	13.6(0.4)	3.1(<0.1)	-16.6(0.4)	3.3(0.10
	MP$_{30-39}$	34.3	31	14.0(0.5)	3.2(<0.1)	-16.3(0.3)	3.4(0.1)
	MP$_{40-49}$	44.9	22	14.6(0.6)	3.2(<0.1)	-16.5(0.7)	3.6(0.2)
	MP$_{50-59}$	55.1	26	14.8(0.7)	3.3(<0.1)	-16.5(0.3)	3.6(0.2)
	MP$_{60-69}$	61.7	16	15.4(0.6)	3.4(0.4)	-16.8(0.8)	3.8(0.2)
	MP$_{70+}$	74.8	5	15.1(1.1)	3.2(0.1)	-16.0(0.2)	3.7(0.3)

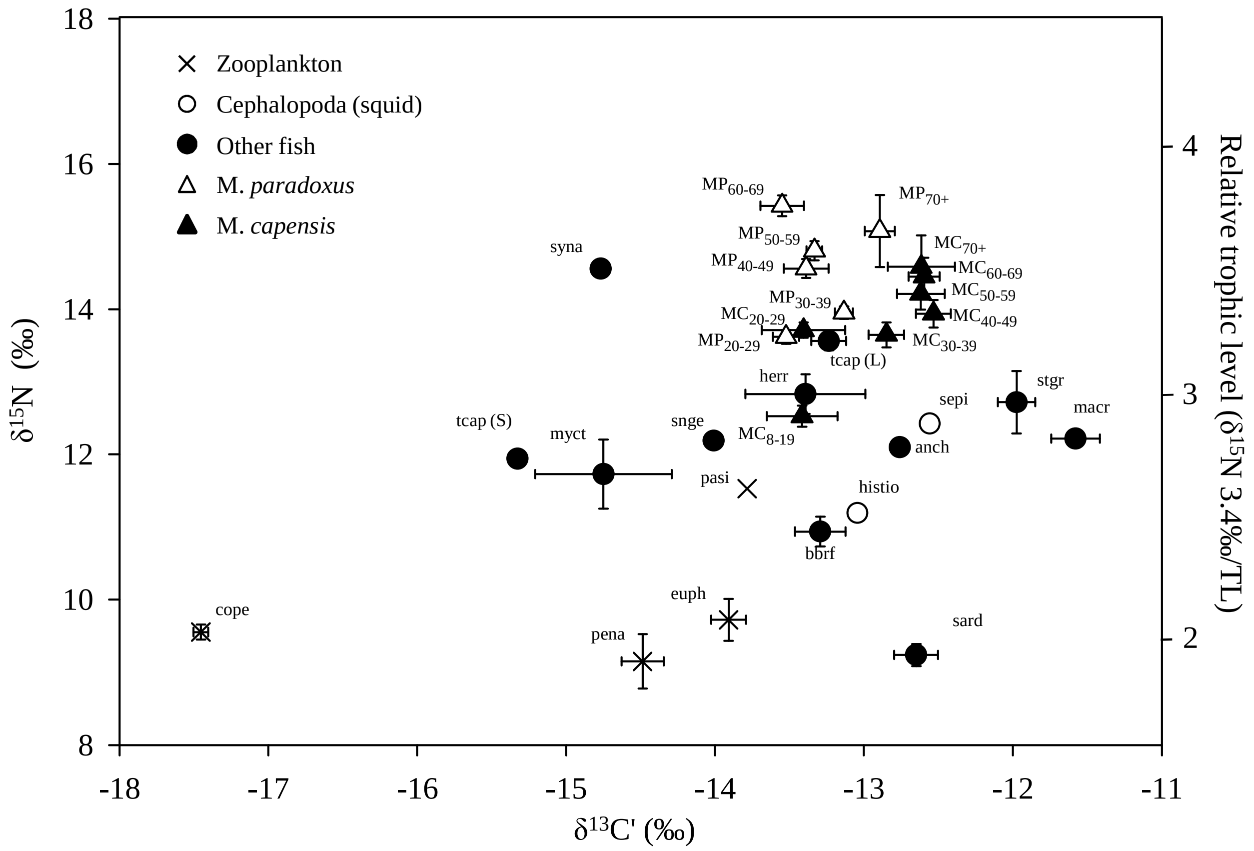

Figure 3.3: Mean (±SE) δ^{15}N and δ ^{13}C by relative trophic level (TL) for hake, potential zooplankton and nekton prey. Species abbreviations are shown in Table 3.1. Subscript number ranges in *Merluccius capensis* ($\text{MC}_{\text{x-y}}$) and *Merluccius paradoxus* ($\text{MP}_{\text{x-y}}$) denote range in total length (cm) of individuals measured.

3.4 Discussion

This study represents the first trophic analysis of the two hake species within the Benguela Current ecosystem using stable isotopes $\delta^{13}C$ and $\delta^{15}N$. Both *M. paradoxus* and *M. capensis* showed significant size-specific shifts in $\delta^{15}N$; however, the overall differences in $\delta^{15}N$ and the slopes of the shift varied between the two species. Smaller hake (<30 cm) were indistinguishable using their $\delta^{13}C$ and $\delta^{15}N$ signatures, suggesting that these size classes shared some degree of spatial and trophic overlap, with larger fish (>30 cm) expressing greater differentiation in both signatures. The smallest individuals of *M. capensis* (8-19 cm) were approximately 3.4‰ (1.1 TL) above the baseline of 9.2‰ (from Kohler *et al.* 2011) and close to the TLs of copepods, penaeid shrimp and euphausiids ($\delta^{15}N$ = 9.6-9.2‰), indicating almost entirely zooplanktivory. For *M. capensis*, there appears to be a large trophic shift in $\delta^{15}N$ from the smallest sizes of 8-19 cm to the next size group of 20-29 cm, and size groups of both *M. capensis* and *M. paradoxus* >20 cm total length were all within the range of 3.3 and 3.8 TLs (Table 3.1).

The general pattern in $\delta^{15}N$ matches well with a comprehensive stomach content study of the two species by Roel and Macpherson (1988) off Namibia, in which they observed differences in diets between larger classes of *M. capensis* and *M. paradoxus*, and a general progression toward piscivory in larger individuals of both species. A significantly enriched ^{15}N values in *M. paradoxus* compared to *M. capensis* was observed, which is similar to Roel and Macpherson's (1988) observation of greater piscivory by the former. By assuming that TLs of 3.0 and 4.0 represent complete zooplanktivory and piscivory, respectively, *M. capensis* and *M. paradoxus* with TLs of 3.3 to 3.8 in this study represent between 30 and 80% piscivory. The size categories between 20-39 cm in *M. capensis* (TL 3.3) and *M. paradoxus* (TL 3.3-3.4) therefore both express a percentage piscivory of around 30-40%, which approximate to Roel and Macpherson's (1988) values of about 25-45% (based on % frequency in the stomachs). In larger size classes of +60 cm from my study, levels of piscivory were 50-60% in *M. capensis* and 70-80% in *M. paradoxus*, which are somewhat below the values of 70-85% in *M. capensis,* but similar to 80-90% in *M. paradoxus*, as published in Roel and Macpherson(1988). The reasons for these differences between our studies seem inherent from the trophic behaviour of *Merluccius* spp. in general, with feeding being highly opportunistic and showing spatial and temporal variability (Roel and Macpherson 1988; Buckley and Livingston 1997; Garrison and Link 2000b; Ferraton *et al.* 2007). Differences were also expected because gut content studies are prone to temporal biases from short feeding events and differential digestion rates of prey (Cortés 1997). On the other hand, stable isotopes integrate what has been synthesized into tissue over a period of time of days to years (Hesslein *et al.* 1993; Miller 2006). Another potential error in my estimations of piscivory by hake could arise from their consumption of sardines, a prey with $\delta^{15}N$ similar to the zooplankton. Roel and Macpherson (1988) listed 14 families of hake prey, but did not include clupeids (sardines), therefore suggesting that sardines are probably not important contributors to the diets of *M. capensis* or *M. paradoxus*.

Diet-analyses of *Merluccius* spp. have shown ontogenetic shift trends from zooplanktivory to piscivory (Bozzano *et al.* 1997; Carpentieri *et al.* 2005), which is consistent with the findings of this study. Le Loc'h and Hily (2005) examined size-specific shifts in $\delta^{15}N$ and $\delta^{13}C$ signatures in European hake (*Merluccius merluccius*) and observed a significant positive relationship in both isotopes with fish size, and estimated size-specific trophic levels of 3.2 in smallest sizes (9-15 cm), 3.7 in medium (19-36 cm) and 4.0 in largest (39-47 cm). Trophic levels of ≥4.0 suggested almost complete piscivory, values not obtained in this study (maximum TL 3.8, *M. paradoxus*) or in other isotope studies of hake from the southern Humboldt Current (trophic level 3.5, *M. gayi*; Hückstädt et al., 2007), and northern California Current (trophic level 3.4, *M. productus*; (Miller *et al.* 2010). This variation may be a result of the underlying differences in trophic structure between ecosystems, such as the relative importance of certain prey (e.g. euphausiids and decapods) associated with upwelling ecosystems compared to regions with significantly lower levels of primary and secondary production. Comparisons of my isotope results with those in van der Bank *et al.* (2011), a study from the same region and time as my hake collections, but one year prior to my prey collections, showed similar $\delta^{15}N$ values in euphausiids (range ~9.0-9.5‰). However, van der Bank *et al.* (2011) reported very different $\delta^{13}C$ values for euphausiids (-16.8‰) compared with mine (~-19.4‰). Copepods from all size classes by van der Bank *et al.* (2011) showed $\delta^{15}N$ ~1.4‰ below my measurement of copepods. For zooplankton it is difficult to compare the two studies because of differences in the way samples were treated. van der Bank *et al.* (2011) lipid-extracted their zooplankton samples prior to measuring $\delta^{15}N$, and this may cause some difference in the isotopic values derived. Furthermore, some prey taxa from my study were obtained through stomach contents of hake, which may have some effect on isotope signatures. In terms of nekton, after adjusting for trophic enrichment ($\delta^{15}N$ = 3.4‰ per TL), comparison of $\delta^{15}N$ and $\delta^{13}C$ measures of pelagic goby (*Sufflogobius bibarbatus*) size classes (van der Bank et al. 2011) to *M. capensis* and *M. paradoxus* size classes suggests substantial trophic interaction, with the small (total length <5.7 cm) and medium (total length 5.8-9.0 cm) sizes of gobies well-matched to isotopic values of *M. capensis* ≥50-69 and *M. paradoxus* ≥40-49 cm total length; $\delta^{15}N$ values of large gobies (>9.0 cm) were well above my highest values in even the largest hake, thus suggesting little or no contribution. These results are only evidential and further analysis by description of diet contents of both hake species is needed to substantiate trophic interactions between hake and pelagic gobies within this system.

Variations in both $\delta^{15}N$ and $\delta^{13}C$ can occur between benthic-pelagic production (e.g. Takai *et al.,* 2002; Nguyen *et al.,* 2011) and between coastal and offshore waters (Miller *et al.* 2008). From multiple linear regression results of hake $\delta^{15}N$ values (within-species), no effects of depth (shelf-slope proxy) or latitude were observed, even after accounting for size. This result suggests that baseline $\delta^{15}N$ values probably did not vary enough to be expressed in higher trophic level organisms. Other evidence supporting spatial homogeneity of isotopes is that very intensive and persistent upwelling in this region can push nearshore upwelled water >150-200 km offshore (Duncombe Rae 2005)**,** and that nearshore-offshore $\delta^{15}N$ and $\delta^{13}C$ measures of surface sediments off Namibia have been shown to be relatively constant from the shelf to as far as >150 km offshore (Holmes *et al.* 1998). Holmes et al., 1998, and Pichevin et al., 2005 **,** however, did observe progressively more enriched $\delta^{15}N$ values in surface sediments from the northern range of my study southward to Lüderitz (average $\delta^{15}N$ ~ 2.0 ‰ higher). To what extent this difference in $\delta^{15}N$ is integrated in the food web from the northernmost area of my study is difficult to ascertain, and it would require more detailed examination of the trophic spectrum from that region. Therefore a caveat in this study is the possibility that relative trophic positions of certain prey species (Table 1) to that of hake and several other taxa may differ in the northern range of my study area.

From $\delta^{13}C$ values of both hake species, only *M. capensis* expressed a significant increase with size and after accounting for size and comparing from the same total depths of collection, on average *M. capensis* expressed more enriched $\delta^{13}C$ values (Fig. 3.3). The large range of $\delta^{13}C$ values has commonly been attributed to a respective influence from benthic and pelagic production in marine systems (e.g., France, 1995; Hobson et al., 1995) but also as an indicator of near shore (enriched ^{13}C) and offshore production (e.g., Miller *et al.*, 2008; Kline, 2009). The two hake species exhibit well known shelf-slope distributions where *M. capensis* (‘shallow water' Cape hake) resides predominantly on the shelf in waters 150-450 m depth (Bianchi *et al.* 1999), with *M. paradoxus* ('deep water' Cape hake) existing further off the shelf-slope (>400 m depth, Lloris *et al.,* 2005). Given Holmes et al. (1998) measurements of constant δ^{13}C in surface sediments 150-200 km offshore of Namibia, and the apparent lack of within-species trend of δ^{13}C with depth in hake (Fig. 3.2), the species-specific differences in δ^{13}C may be more attributed to their proximity to benthic production. That is, shallow water hake live in closer proximity to the benthos and therefore have a greater relative dependence on benthic-based production than *M. paradoxus,* which is in deeper water and probably consumes more pelagic-mesopelagic prey. The precise measurement of δ^{13}C-based benthic and pelagic production is difficult to obtain in this system because very high upwelling and primary production within the region can create substantial deposition of pelagic production to sediments, which can be re-suspended and reintegrated into both benthic and pelagic production. Evidence of this effect off Namibia is the poor discrepancy in δ^{13}C benthic and pelagic organisms (van der Bank 2011), in which benthic polychaetes and sediments were equal to or lower than the δ^{13}C values of pelagic consumers within their study, but also from those reported here.

Relative to hake δ^{13}C values, the δ^{13}C signatures of prey were too constrained to allow for assessment of sources with isotope mixing models that use carbon isotope only. Both *M. paradoxus* and *M. capensis* are generalists and therefore are a mixture of diverse prey from nearshore-offshore and benthic-pelagic habitats. The relative values from δ^{15}N, however, were useful in revealing the degree of trophic overlap of these species relative to the trophic position of fish and invertebrate prey. Future studies may use the isotope information herein as a benchmark for monitoring trophic shifts in the northern Benguela Current, which has experienced significant shifts in nekton and zooplankton community structure over the last several decades (Watermeyer *et al.* 2008). It is interesting to note that the δ^{13}C values that I measured in the overall food web off Namibia appear considerably more enriched (less negative) than those observed in other coastal food webs such as the continental shelves off Newfoundland and Labrador (Sherwood and Rose 2005) and south-eastern Australia (Davenport and Bax 2002). Enriched δ^{13}C values can be indicative of a greater diatom-based production in marine food webs (Miller *et al.* 2008) and my enriched δ^{13}C values correspond well with the very high degree of upwelling-driven diatom production off Namibia (Romero *et al.* 2002).

CHAPTER 4: DIETARY CONTRIBUTIONS OF DIFFERENT PREY TO THE DIETS OF HAKE (*MERLUCCIUS CAPENSIS* AND *M. PARADOXUS)* OFF NAMIBIA: ISOTOPE MIXING MODEL APPROACH

4.1 Introduction

Predators can feed on the most abundant prey in their surroundings (Mesa *et al.* 1994) or they can choose specific prey from those available (Schlacher and Wooldridge 1996). Feeding ecology of exploited fish species has seldom been considered in evaluating their population dynamics in fisheries management (Jaworski and Ragnarsson 2006). General consensus has emerged among fisheries scientists that fish feeding ecology represents a major aspect of food web linkages, and it warrants consideration when managing fisheries resources (Gascuel *et al.* 2005; Shannon *et al.* 2010)**.**

The two species of hake (*Merluccius capensis* and *M. paradoxus*) are important predators in marine waters off Namibia (Macpherson and Roel 1987; Roel and Macpherson 1988). Early attempts to look at their diets in the Benguela Current ecosystem indicated that hake feed on a variety of fish and crustaceans (Assorov and Kalinina 1979). Payne et al. (1987) reported that crustaceans, mesopelagic fish and hake are the most important in the majority of length classes of hake studied. In the marine waters off Namibia, Roel and Macpherson(1988) described hake diet as consisting of mainly small crustaceans, fish and cephalopods. On the west coast of South Africa hake fed mainly on crustaceans, with anchovy being the dominant dietary item of juvenile *M. capensis*, a reflection of the increased availability of anchovy at the time of the surveys (Punt *et al.* 1992). At sites abundant in zooplankton, hake fed preferentially on large crustaceans [amphipods, euphausiids and shrimp (*Pasiphaea semispinosa*)], while at a site dominated by recruits of anchovy (*Engraulis capensis*), the hake were almost exclusively piscivorous (Pillar and Barange 1993).

On the south coast of South Africa, fish remains in hake stomach contents accounted for 92% of prey by mass, 5% for crustaceans and 3% for cephalopods, contrasting with that documented for the West Coast of south Africa (Pillar and Wilkinson 1995). For small hake, especially *M. paradoxus*, euphausiids were consumed more frequently in summer and mesopelagic fish were of more dietary importance in winter, with hake-on-hake predation a more prominent feature in *M. capensis* (Pillar and Barange 1997). Macpherson and Gordoa (1994) observed that in large *M.*

capensis, cannibalism is not related to the density of the small conspecifics, nor to the density of alternative prey, but rather that large hake have a dietary preference for small conspecifics. The most recent study of hake diet off Namibia, indicated that crustaceans and cephalopods were of minor importance compared to fish in *M. capensis*, but they contributed relatively more to the diet of *M. paradoxus* (Traut 1996). The same study also found hake was a more important prey item for *M. capensis* than for *M. paradoxus*.

The above cited studies showed that the relative importance of prey in the diets of the two hake species varies both seasonally and geographically. The opportunistic nature of hake feeding, which changes with prey availability, plays a major role in determining contributions of prey to their diets. The use of stomach content analyses in studying these dynamics however, highlighted the difficulties in relating results of feeding patterns of hake from different time periods and locations.

The increased use of stable isotopes (δ^{15}N and δ^{13}C) in trophic research (Takai *et al.* 2007; Stowasser *et al.* 2009; Iitembu *et al.* 2012) has led to the development of isotope mixing models (Phillips and Gregg 2003; Parnell *et al.* 2010). These mixing model approaches are based on the idea that a predator's tissues are an isotopic mixture of its dietary sources and can indicate the proportional contributions of these sources to its diet (Phillips and Gregg 2003; Parnell *et al.* 2010). Feasible contributions of different prey to the isotopic mixture of the predator's tissues have been used as a proxy of their dietary contributions (Boyle *et al.* 2012; Hopkins III and Ferguson 2012; Quevedo *et al.* 2012). Results from mixing models reduce biases associated with stomach content analyses because of the use of time and space-integrated isotopic data (Layman and Allgeier 2012).

This study contributes to the research efforts of understanding the contributions of different prey to the hake diet off Namibia (*M. capensis* and *M. paradoxus*). I used an isotope mixing model to determine the feasible contributions of prey to the isotopic mixture of hake and assessed how it varied with fish growth (length). I hypothesized that the temporally integrated dietary representations of the two species diets are similar, given that they both feed on small fishes and crustaceans.

4.2 Methods and materials

4.2.1 Field sampling

All hake and prey samples were collected during a hake biomass survey as described in Chapter 2, section 2.1.

4.2.2 Laboratory analysis

All hake and prey samples were measured for $\delta^{13}C$ and $\delta^{15}N$ at IsoEnvironmental CC Laboratory at Rhodes University (Grahamstown, South Africa) as described in Chapter 2, section 2.2.1. The relative trophic positions of all the species were calculated as describe in Chapter 2, section 2.2.1.

4.2.3 Statistical analysis

Mean isotopic composition and its standard deviation (SD) were calculated for hake species (*M. capensis* and *M. paradoxus*) and their prey. The significance differences among $\delta^{15}N$ and $\delta^{13}C$ values of the prey species were investigated using an analysis of variance (ANOVA) test with a Tukey HSD (Tukey Honest Significant Difference) *post hoc* test to perform multiple comparisons. Data were examined for normality and homogeneity of variance using a Shapiro–Wilk and Levene test. Violations of homogeneity and normality were addressed through log transformations. A Bayesian stable-isotope mixing model, termed Stable Isotope Analysis in R (SIAR) (Parnell *et al.* 2010),was used to obtain the feasible contributions of the different species to the isotopic signatures of the two hake species (*M. capensis and M. paradoxus*). Isotopic signatures and fractionation together with the uncertainties of these values were included in the model (Parnell *et al.* 2010). By defining a set of N mixture measurements on *J* isotopes with K source contributors, the mixing model is expressed by Parnell *et al.* (2010) as:

$$X_{ij} = \frac{\sum_{k-1}^{k} P_k (s_{jk} + c_{jk}) + \varepsilon_{jk}}{\sum_{k-1}^{k} p_k q_{jk}}$$

$$S_{jk} \sim N(\mu_{jk}, \omega_{jk}^2)$$

$$c_{jk} \sim N(\lambda_{jk}, \tau_{jk}^2)$$

$$\varepsilon_{ij} \sim N(0, \sigma_j^2)$$

X_{ij} = observed isotope value j of the consumer i, s_{jk}= source value k on isotope j; normally distributed with mean $\mu_{jk}$and variance ω_{jk}^2, C_{jk} = fractionation factor for isotope j on source k; normally distributed with mean λ_{jk} and variance $\tau_{jk,}^2$ P_k= dietary proportion of source k (estimated by the model), q_{jk} = concentration of isotope j in source k, ε_{ij} = residual error, describing additional inter- observation variance not described by the model and σ_j^2 is estimated by the model. A detailed description of this model can be found in Parnell et al. (2010) and compared to other mixing models in Galva et al. (2012).

The fractionation factors of 3.2 ±1.28‰ for $\delta^{15}N$ (Sweeting *et al.* 2007b) and 1.56±1.10‰ for $\delta^{13}C$ (Sweeting *et al.* 2007a) were used. In addition to a number of hake prey species (see Table 4.1), hake (*M. capensis* and *M. paradoxus*) of ≤ 40 cm were also used as prey to account for cannibalism. The number of prey were chosen based on accounts of published hake stomach content results (Payne *et al.* 1987; Roel and Macpherson 1988; Traut 1996). The model was run using R package SIAR (R Development Core Team 2012, Vienna Austria).

4.3 Results

4.3.1 Isotopic characteristics of the prey

Samples from 299 individuals of fish, 16 marine species were analysed to derive their stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$). Crustaceans (5 species), teleost fish (9 species) and cephalopods (2 species) were used as prey for the two hake species (Table 4.1). *Merluccius capensis* sizes ranged from 18 cm to 69 cm, while *M .paradoxus* sizes ranged from 22cm to 65 cm. The prey species sizes ranged from the smallest crustaceans (euphausiids*)* of 4 cm to a teleost (*Nezumia micronychodon)* of 24 cm. Among the prey considered, a teleost (*N. micronychodon***)** had the most enriched $\delta^{15}N$ (14.87‰), while *C. agazizi* had the most depleted $\delta^{15}N$ (9.84‰) (Table 4.1). The prey relative trophic levels ranged from 1.99 for C. *agazizi* to 3.4 for *N. micronychodon* (Table 4.1).

To determine whether isotopic signatures were different among potential prey, two ANOVA were run (one for nitrogen and one for carbon), followed by Tukey HSD *post hoc* tests to identify any specific differences. There was a significant difference among species' $\delta^{15}N$ (ANOVA, $p < 0.05$, F (17,131) =8.81). In term of $\delta^{15}N$, *N. micronychodon* was significantly different from all the prey species ($p<0.05$). *Epigonus denticulatus* was significantly different from *Funchalia woodwardi* (p<0.001), euphausiids (p<0.001) and *Plesionika martia* (p<0.001). Euphausiid nitrogen signatures were significantly different from those of *Emmelichthys nitidus nitidus* (p=0.003), *Phosichthys argenteus* (p=0.03) and *Paracallionymus costatus* (p=0.009) and *Plesionika martia* was significantly different from small hake (p=0.002), *P. costatus* (p=0,001), *P. argenteus* (p=0.007) and *Todarodes sagittatus* (p=0.03).

There was a significant difference among species' δ^{13}C (ANOVA, $p < 0.05$, F (17,131) =2.66). In terms of δ^{13}C of prey species, C. *capensis* had the most enriched δ^{13}C (17.84‰) while *P. argenteus* had the lowest δ^{13}C (13.96‰) (Table 4.1). ANOVA followed by a Tukey HSD *post hoc* test indicated that *C. capensis* was significantly different from *F. woodwardi* (p=0.013), *E. denticulatus* (p<0.001), *E. nitidus nitidus* (p=0.001), euphausiids (p=0.002), myctophids (p<0.001), *P. argenteus* (p<0.001), *P. costatus* (p<0.001), *P. martia* (p<0.001) and small hake [<15 cm, p=0.008)]. Myctophids were significantly different from *A. varidens* (p<0.001), *P. argenteus (P=0.02)*, *F. woodwardi* (p<0.001), *Helicolenus dactylopterus* (p<0.001), euphausiids (p=0.01), *N. micronychodon* (p<0.001) and *Solenocera africana* (p<0.001). *Phosichthys argenteus* was significantly different from *Aristeus varidens* (p=0.02), *N. micronychodon* (p=0.003) and *S. africana* (p=0.002). *Plesionika martia* was significantly different from *S. africana* (p=0.003).

Table 4.1: Summary mean (±SD) values of nitrogen and carbon stable isotopes of species from the study, with respective sample sizes(n), L (average length), $\delta^{13}C_n$ (lipid normalized) and standard deviation, C: N ratios and trophic level (TL).

Prey categories	**Family**	***n***	**L(cm)**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{13}C_n$**	**C:N**	**TL**
Crustaceans								
Aristeus varidens	Aristeidae	19	9	11.80 (1.05)	-16.16(0.99)		3.15	2.57
Solenocera africana	Solenoceridae	10	7	11.71 (1.09)	-15.66 (0.07)		3.13	2.55
Plesionika martia	Pandalidae	19	7	10.71 (1.09)	-17.16 (0.94)		3.15	2.25
euphausiids(krill)	Euphusidae	16	4	10.91 (0.56)	-16.93 (0.24)		3.28	2.33
Funchalia woodwardi	Penaeidae	16	8	11.70 (1.70)	-16.58 (1.20)		3.15	2.54
Teleosts								
Chelidonichthys capensis	Triglidae	6	23	11.09(0.56)	-13.96 (0.38)		3.25	2.93
Chlorophthamus agazizi	Chlorophthalmidae	5	11	9.84 (2.72)	-16.38 (0.73)		3.28	1.99
Emmelichthys nitidus nitidus	Emmelichthydae	4	21	13.02(0.25)	-17.23 (0.27)		3.44	3.22
Epigonus denticulatus	Epigomidae	5	16	14.05 (0.19)	-16.81(0.17)		3.23	2.83
Etrumeus whiteheadi	Clupeidae	8	20	12.67(0.33)	-16.82 (0.02)		3.46	2.83
Helicolenus dactylopterus	Scorpaenidae	4	15	12.20 (2.12)	-16.09 (1.72)		3.19	2.69
myctophids	Myctophidae	12	5	12.62(0.51)	-18.99 (0.47)	-15.60(0.48)	6.78	2.81
Nezumia micronychodon	Macrouridae	5	24	14.87(0.60)	-15.82 (0.44)		3.12	3.40
Paracallionymus costatus	Callionymidea	5	24	13.52(0.32)	-17.43(0.62)	-17.07(0.55)	3.71	3.04

Table 4.1. continued

Phosichthys argenteus	Phosichthyidae	11	17	12.83(0.78)	-17.84(0.28)		3.08	2.87
Sufflogobius bibartus	Gobidae	5	11	12.74 (0.45)	-15.62 (0.32)		3.39	2.85
Synagrops microlepis	Acropomatidae	26	9	10.21(0.84)	-16.94(1.84)	-15.81(0.58)	4.50	2.10
small hake (<15 cm)	Merluccidae	10	12	12.41(0.28)	-16.73 (0.42)		3.23	2.74
M. paradoxus (<40 cm)	Merluccidae	26	33	13.06(0.64)	-16.65(0.5)		3.20	2.94
M. capensis(<40 cm)	Merluccidae	34	27	12.87(0.82)	-16.62(1.0)		3.19	2.88
M. paradoxus (>40 cm)	Merluccidae	26	48	13.57(0.42)	-16.58 (0.56)		3.31	3.10
M. capensis(>40 cm)	Merluccidae	19	52	13.26 (0.74)	-15.76(0.52)		3.23	3.02
Cephalopods								
Todarodes sagittatus	Ommastrephidae	2	44	12.79(0.88)	-16.70(0.24)		3.26	2.86
Todaropsis eblanae	Ommastrephidae	6	13	11.95(0.52)	-17.10(0.47)		3.43	2.61

4.3.2 Prey dietary contributions

Feasible contributions of different prey to the isotopic mixtures of the two hake species (all size-pooled) showed dietary overlap between the hake (Table 4.1). For both species, *S. microlepis* was the highest feasible contributor to their isotopic mixtures, with credible intervals of 4.77-34.24 % and 4.04 -38.68% for *M. paradoxus* and *M. capensis*, respectively. *Plesionika martia*, *C. agazizi*, myctophids and euphausiids had notable feasible contributions to the isotopic mixtures of the two species (Table 4.2). The contributions of small hake (<15 cm) were 0 -4 % for *M. paradoxus* and 0 **-** 6 % for *M. capensis*. The contributions of medium size hake (<40 cm), as prey, averaged 1.21% for *M. paradoxus* and 1.6% for *M. capensis.* The contribution of small and medium size hake represented cannibalism of less 6 % and 4%, for *M. capensis* and *M. paradoxus* respectively (Table 4.2).

The contributions of different prey species per length classes indicated that *P. martia*, *C. agazizi*, myctophids and euphausiids were dominant contributors to both hake species (Tables 4.3 & 4.4). For hake in the 20-29 cm length class, myctophids contributed more than all other prey (modal contributions of 6.72 % for *M. capensis* and 6.78 % for M. *paradoxus).* In all length classes of *M. capensis*, myctophids had higher modal contributions than all prey, while for *M. paradoxus* larger than 30 cm, *Synagrops microlepis* was the highest contributor to the isotopic mixture.

In hake between 20 -29 cm, three crustacean species (*P. martia*, euphausiids, and *F. woodwardi)* had modal contributions of more than 3% for *M. paradoxus*. For *M. capensis*, only *P. martia* and euphausiids had modal contributions more that 3% at 20-29 cm length classes. Of the cephalopods, *Todaropsis eblanae* had modal contributions of more than 3 % in the length classes of 30-39 cm for *M. capensis* and 20-29 cm for *M. paradoxus*.

Helicolenus dactylopterus had the widest 95% credible interval (CI) of their dietary feasible contributions (0-11.18 %) for *M. paradoxus* in 20-29 cm length class. In 30-39 cm length class, myctophids had the widest CI (0.0-15.92 %) while in 40-49 cm length class, C. *agazizi* had the CI (0.0-17.68 %) and in >50 cm length class, S. *microlepis* had the CI (0.00-10.70 %). Myctophids had the widest CI of their feasible contributions at all length classes of *M. capensis*. Small hake (<15 cm), *M. paradoxus* (<40 cm) *and M. capensis* (<40 cm) all had modal contributions of around 1% in all length classes for both species, with their upper level CI around 9 % (Tables 4.3 & 4.4).

Table 4.2: Mode, mean and 95 % credible intervals (CI) of feasible contributions of the prey categories into the isotopic mixtures of *M. capensis* and *M. paradoxus,* respectively; all sizes of hake were pooled.

	M. paradoxus				***M. capensis***			
	Mode	**Mean**	**95% CI**		**Mode**	**mean**	**95 %CI**	
Crustaceans			**Low**	**High**			**Low**	**High**
Aristeus varidens	0.63	1.82	0.00	4.83	0.49	2.24	0.00	6.79
Solenocera africana	0.59	1.48	0.00	3.98	0.79	2.08	0.00	5.11
Plesionika martia	9.38	12.08	1.51	25.58	10.42	13.28	0.94	23.55
euphausiids(krill)	9.26	7.77	0.00	16.80	7.70	7.76	0.04	15.30
Funchalia woodwardi	0.83	2.50	0.00	5.72	0.72	3.37	0.00	8.81
Teleosts								
Chelidonichthys capensis	0.74	1.13	0.00	2.56	0.41	1.30	0.00	3.63
Chlorophthamus agazizi	18.54	18.32	4.24	30.88	13.92	15.71	1.41	27.70
Emmelichthys nitidus nitidus	0.23	1.54	0.00	3.75	0.62	2.38	0.00	6.77
Epigonus denticulatus	0.99	1.22	0.00	2.99	0.27	1.19	0.00	3.41
Etrumeus whiteheadi	0.54	1.70	0.00	4.80	0.80	2.08	0.00	5.65
Helicolenus dactylopterus	0.46	1.36	0.00	3.78	0.32	1.74	0.00	4.91
myctophids	5.09	14.30	0.00	36.10	10.03	11.19	0.17	22.76
Nezumia micronychodon	0.23	0.64	0.00	1.49	0.27	0.79	0.00	2.13
Paracallionymus costatus	0.38	1.59	0.00	4.61	0.83	1.89	0.00	4.99
Phosichthys argenteus	0.74	3.25	0.00	8.81	0.76	3.34	0.00	8.65
Sufflogobius bibartus	0.19	1.03	0.00	2.37	0.32	1.29	0.00	3.76
Synagrops microlepis	19.34	19.56	4.77	34.24	16.74	17.20	4.04	28.68
small hake (<15 cm)	0.46	1.71	0.00	4.12	1.01	2.16	0.00	6.16
M. paradoxus (<40 cm)	0.25	1.20	0.00	3.38	0.36	1.61	0.00	4.76
M. capensis(<40 cm)	0.23	1.31	0.00	3.28	0.40	1.67	0.00	4.96
Cephalopods								
Todarodes sagittatus	0.31	1.65	0.00	4.25	0.34	1.87	0.00	5.31
Todaropsis eblane	0.64	2.85	0.00	7.69	1.07	3.86	0.00	9.31

Table 4.3: Mode and 95 % credible intervals (CI) of feasible contributions of the prey in the isotopic mixtures of M. *paradoxus* in different length classes

M. paradoxus	**20-29 cm**			**30-39 cm**			**40-49 cm**			**>50 cm**		
	Mode	**95% CI**		**Mode**	**95% CI**		**Mode**	**95% CI**		**Mode**	**95% CI**	
Crustaceans		**Low**	**High**		**Low**	**High**		**Low**	**High**		**Low**	**High**
Aristeus varidens	1.22	0.00	9.44	1.04	0.00	9.79	1.04	0.00	9.76	1.17	0.00	9.63
Solenocera africana	1.42	0.00	9.22	0.91	0.00	9.03	0.92	0.00	8.88	1.08	0.00	9.38
Plesionika martia	5.49	0.00	10.69	6.63	0.00	13.84	6.73	0.00	16.03	6.21	0.00	10.46
Euphausiids(Krill)	4.54	0.00	10.26	3.98	0.00	12.75	6.37	0.00	14.83	5.12	0.00	10.23
Funchalia woodwardi	3.51	0.00	9.74	1.31	0.00	10.63	1.12	0.00	10.99	1.62	0.00	9.92
Teleosts												
Chelidonichthys capensis	0.86	0.00	8.50	0.75	0.00	7.68	0.70	0.00	7.29	0.98	0.00	9.06
Chlorophthamus agazizi	4.22	0.00	9.71	6.05	0.00	14.06	6.94	0.00	17.68	6.33	0.00	10.65
Emmelichthys nitidus nitidus	1.54	0.00	9.24	1.11	0.00	9.97	1.04	0.00	9.52	1.94	0.00	9.55
Epigonus denticulatus	1.13	0.00	9.67	0.76	0.00	8.17	0.70	0.00	7.11	0.94	0.00	9.08
Etrumeus whiteheadi	1.19	0.00	9.23	1.05	0.00	9.68	0.95	0.00	9.21	1.33	0.00	9.50
Helicolenus dactylopterus	0.98	0.00	11.88	0.93	0.00	8.95	0.90	0.00	9.07	1.07	0.00	9.31
Myctophids	6.78	0.00	8.39	7.22	0.00	15.92	7.55	0.00	16.35	5.95	0.00	10.52
Nezumia micronychodon	0.92	0.00	9.66	0.59	0.00	6.50	0.49	0.00	5.36	0.91	0.00	8.52
Paracallionymus costatus	1.40	0.00	10.34	1.05	0.00	9.55	0.85	0.00	8.96	1.08	0.00	9.33
Phosichthys argenteus	4.57	0.00	8.89	1.60	0.00	11.55	1.34	0.00	11.04	1.53	0.00	9.87
Sufflogobius bibartus	1.06	0.00	10.76	0.77	0.00	7.90	0.74	0.00	7.23	0.85	0.00	9.04
Synagrops microlepis	5.59	0.00	9.58	7.32	0.00	14.70	8.11	0.04	17.82	6.21	0.00	10.70

Table 4.3 continued												
Small hake (<15 cm)	1.01	0.00	9.32	1.12	0.00	9.79	0.96	0.00	9.72	1.23	0.00	9.50
M. paradoxus (<40 cm)	1.10	0.00	9.25	0.96	0.00	8.89	0.87	0.00	8.33	1.04	0.00	9.21
M. capensis(<40 cm)	1.42	0.00	10.30	1.05	0.00	9.08	0.91	0.00	8.57	1.97	0.00	9.20
Cephalopods												
Todarodes sagittatus	1.21	0.00	9.53	1.08	0.00	9.34	0.83	0.00	8.74	1.76	0.00	9.25
Todaropsis eblane	3.15	0.00	10.08	1.58	0.00	11.20	1.97	0.00	11.50	4.09	0.00	9.84

Table 4.4: Mode and 95 % credible intervals (CI) of feasible contributions of the prey in the isotopic mixtures of *M. capensis* in different length classes

M. capensis	**20-29 cm**			**30-39 cm**			**40-49 cm**			**>50 cm**		
	Mode	**95% CI**		**Mode**	**95% CI**		**Mode**	**95% CI**		**Mode**	**95% CI**	
Crustaceans		**Low**	**High**		**Low**	**High**		**Low**	**High**		**Low**	**High**
Aristeus varidens	1.00	0.00	9.86	1.05	0.00	9.41	1.33	0.00	9.30	1.08	0.00	9.32
Solenocera africana	1.03	0.00	9.03	0.92	0.00	8.74	0.98	0.00	8.95	0.96	0.00	9.09
Plesionika martia	6.34	0.00	13.69	5.67	0.00	12.60	4.82	0.00	11.30	5.93	0.00	12.38
Euphausiids(Krill)	5.66	0.00	12.70	4.86	0.00	11.92	3.48	0.00	10.80	5.68	0.00	11.31
Funchalia woodwardi	1.15	0.00	10.47	1.17	0.00	10.26	1.62	0.00	10.19	1.11	0.00	10.16
Teleosts												
Chelidonichthys capensis	0.80	0.00	7.96	0.65	0.00	7.41	0.76	0.00	7.49	0.72	0.00	7.66
Chlorophthamus agazizi	5.10	0.00	13.96	3.82	0.00	11.83	3.96	0.00	10.45	4.39	0.00	11.31

Table 4.4 continued

Emmelichthys nitidus nitidus	1.14	0.00	9.68	1.14	0.00	9.92	1.16	0.00	10.20	1.12	0.00	10.08
Epigonus denticulatus	0.81	0.00	8.22	0.94	0.00	8.92	1.13	0.00	9.26	0.99	0.00	8.93
Etrumeus whiteheadi	0.93	0.00	9.77	1.13	0.00	9.60	1.63	0.00	9.69	1.15	0.00	9.67
Helicolenus dactylopterus	1.00	0.00	9.15	0.91	0.00	9.23	1.03	0.00	9.21	1.09	0.00	9.18
Myctophids	6.72	0.00	14.48	7.31	0.00	16.02	7.24	0.13	12.96	7.38	0.00	14.57
Nezumia micronychodon	0.64	0.00	6.68	0.72	0.00	7.38	0.93	0.00	8.14	0.78	0.00	7.65
Paracallionymus costatus	0.93	0.00	9.43	1.27	0.00	10.33	1.63	0.00	10.30	2.03	0.00	10.18
Phosichthys argenteus	3.72	0.00	10.96	5.27	0.00	11.82	6.60	0.00	11.05	5.26	0.00	11.13
Sufflogobius bibartus	0.92	0.00	8.21	0.78	0.00	8.18	0.84	0.00	8.47	0.89	0.00	8.45
Synagrops microlepis	6.61	0.00	14.33	6.32	0.00	13.30	5.02	0.00	11.17	5.58	0.00	12.45
Small hake (<15)	0.99	0.00	9.87	1.13	0.00	9.69	1.09	0.00	9.84	1.19	0.00	9.79
M. paradoxus (<40 cm)	0.96	0.00	8.76	0.94	0.00	9.22	1.05	0.00	9.38	0.99	0.00	9.26
M. capensis(<40 cm)	0.97	0.00	8.97	1.00	0.00	9.22	1.03	0.00	9.54	0.89	0.00	9.34
Cephalopods												
Todarodes sagittatus	0.98	0.00	9.15	0.93	0.00	9.35	1.20	0.00	9.61	1.06	0.00	9.34
Todaropsis eblane	1.37	0.00	11.42	4.75	0.00	10.96	1.48	0.00	10.35	2.09	0.00	10.66

4.4 Discussion

Studies of feeding ecology of the two species of hake (*M. capensis* and *M. paradoxus*) in the Benguela Current region have previously been done using stomach content analyses (Payne *et al.* 1987; Roel and Macpherson 1988; Traut 1996). Estimates of hake feeding using gut contents are imprecise in identifying the relative contributions of different prey because of the opportunistic feeding behaviours of hake (Punt *et al.* 1992), and the natural variability associated with prey densities (Macpherson and Gordoa 1994). In this study, I utilized stable isotope mixing models to assess the contributions of different prey to the isotopic mixture of each hake species. The temporally-integrated estimates of their diets from my results differed from those of previous data derived from stomach content analyses, which appeared to have overestimated the contributions of certain prey to the diets of hake.

Potential prey collected consisted of small fish, crustaceans and cephalopods identified in previous studies incorporating stomach content analyses (Payne *et al.* 1987; Roel and Macpherson 1988; Traut 1996). Prey sizes ranged from the smallest crustaceans (euphausiids*)* of 4 cm to a teleost (*N. micronychodon)* of 24 cm. The range was chosen to cover the size that can potentially be eaten by hake of different sizes, as prey choices by predators in marine ecosystem are influenced by the size of the prey available (Scharf *et al.* 1998; Emmerson and Raffaelli 2004).

Among the prey, *N. micronychodon* had the most enriched δ^{15}N (14.87‰), indicating that it feeds at a higher relative trophic position to all other prey collected in this study. The estimated trophic position of *N. micronychodon* of 3.4 was higher than the average estimated for larger *M. paradoxus* and *M. capensis* (Table 4.1). It is possible that *N. micronychodon,* being the largest prey, is too large to be consumed by the two hake species. However, fish from the Macrouridae family have been found in hake stomachs (Roel and Macpherson 1988), and the feeding range of some hake species (e.g. *Merluccius gayi*) included prey more than half their length (Konchina 1983). *Chlorophthamus agazizi* had the most depleted δ^{15}N (9.84‰), with an estimated trophic position of 1.99.

In terms of δ^{13}C, *C. capensis* had the most depleted ^{13}C (-17.84‰) values, and it represented the pelagic and off-shore ecosystem, as benthic and near shore species have more enriched ^{13}C values relative to those from pelagic and off-shore environments (France 1995b). *Phosichthys* *argenteus,* with the most enriched ^{13}C (-13.96‰) values, represent species from the benthic/near shore environment (Miller *et al.* 2008). Although the difference in^{13}C provided evidence of habitat related carbon source (phytoplankton vs. benthic primary production) separation of ^{13}C values of prey, some of the variations can be from lipid content (Post et al., 2007), and geographical differences in plankton biosynthesis and metabolism (Rau et al., 1989). The ranges of prey δ^{15}N in this study therefore represented multiple trophic positions, while their δ^{13}C ranges represented diverse basal resources.

Some prey were not statistically different from each other in terms of either δ^{13}C or δ^{15}N. The similarities of δ^{13}C and δ^{15}N may be a result of mixing of cross-shelf and offshore waters through upwelling, resulting in homogeneous mixture of food sources available to the various prey. Holmes et al.(1998) observed little change in δ^{13}C and δ^{15}N values in surface sediments up to 200 km off shore. The similarities in the isotopic values among prey may present a problem, as different combinations of prey species can produce the same isotope signatures. However, the ability of the SIAR model to incorporate variations of isotope values reduces the impact of overlapping or similar isotope ratios of prey (Parnell *et al.* 2010).

Feasible contributions of different prey to hake isotopic mixtures (all sizes-pooled) showed that the two hake species' diets overlapped. This confirmed the findings of previous studies using stomach contents (Payne *et al.* 1987; Roel and Macpherson 1988). *Synagrops microlepis* was the highest feasible contributor to both species isotopic mixture, with credible intervals of 4.77-34.24% and 4.04 -38.68% for *M. paradoxus* and *M. capensis*, respectively (Table 4.2). These specific results contrast the previous findings obtained using stomach content analyses, which indicated that crustaceans were the main food for both species (Roel and Macpherson 1988). The differences could be a result of the temporally-integrated nature of isotope data, in which signatures are assimilated over a longer period (Haines 1976; West *et al.* 2006), as opposed to gut contents which are 'snap shots' of recent feeding (Paine 1988). *Plesionika martia, C. agazizi*, myctophids and euphausiids also made considerable contributions to the diets of the two hake species. Results in my study indicated that the diets of the two hakes were not dominated by a single taxon, i.e. fish, a finding similar to that based on gut contents (Roel and Macpherson 1988). Cannibalism accounted for less than 6% and less than 4% in the diets of *M. capensis* and *M. paradoxus,* respectively (Table 4.2). The greater degree of cannibalism in *M.*

capensis confirms patterns observed by Traut (1996). The cannibalism estimates from most studies utilising gut contents(Roel and Macpherson 1988; Macpherson and Gordoa 1994; Traut 1996) were, however, much higher than my own estimates. Cannibalism is possibly overestimated by stomach contents analyses. The two hake species occur in great numbers in the marine waters off Namibia (Burmeister 2001). Prey patch characteristics such as local densities of hake at the times of feeding (Benoit-Bird *et al.* 2013) can, therefore, bias their contributions to overall diet. Payne *et al.* (1987) also concluded that much of the feeding is not true cannibalism because adult *M. capensis* feed largely on small *M. paradoxus* where the latter co-occurs in great numbers**.**

The contributions of prey to the isotopic mixtures of hake (sizes-pooled) might not reflect their correct dietary representations, as fish feeding patterns change during growth (Persson and De Roos 2003; Emmerson and Raffaelli 2004).The ontogenic trophic shift of hake species (Iitembu *et al.* 2012) can result in different prey contributions with different size classes, which necessitated the separation of hake into different length classes. The contributions of different prey to different length classes of hake indicated that for both species, *P. martia*, *C. agazizi*, myctophids and euphausiids are dominant contributors, findings similar to those of the pooled size classes for hake. For small hake (20-29 cm), myctophids contributed more than all other prey, with modal contributions of 6.72 % for *M. capensis* and 6.78 % for M. *paradoxus*. Myctophids had higher modal contributions than all other prey to all length classes of *M. capensis,* which differ from Roel and Macpherson's (1988) findings of myctophid feeding decreasing with hake length**.** Myctophids were dominant only in *M. paradoxus* less than 30 cm, while in large ones, *S. microlepis* had the highest modal contributions to their isotopic mixture. The dietary differences between the two hake species are also shown by the 95% CI, where in *M. paradoxus* of the 20-29 cm length class, *H. dactylopterus* had the widest CI (0-11.18 %). In *M. paradoxus* between 30-39 cm, myctophids had the widest CI (0.0-15.92 %). In the 40-49 cm length class of *M. paradoxus, C. agazizi* had the widest feasible co-contributions (0.0-17.68 %), while in *M. paradoxus* of >50 cm *S. microlepis* had the widest CI (0.00-10.70 %). *Merluccius paradoxus* appears to feed on more diverse prey in comparison to *M. capensis*, and the importance of prey changed as they grew in length. Myctophids had the widest CI in all length classes of *M. capensis*, indicating their importance to the diet of *M. capensis*.

Contributions of crustaceans to the diets of hake indicated that, in the 20 -29 cm length class, *F. woodwardi* with *P. martia* and euphausiids had modal contributions of more than 3% for *M. paradoxus.* For *M. capensis* of the same size, only *P. martia* and euphausiids modal contributions of more than 3%. The above dietary contributions shows that while the smaller hake species are isotopically indistinguishable (Iitembu *et al.* 2012), they differed in terms of dietary contributions of prey species. It is possible for two species feeding on different prey to have similar stable-isotope signatures, especially if the prey consumed have similar isotopic signatures (Thomson *et al.* 2012)**.**

Payne et al. (1987) found cephalopods were important food for hake from a length of 50 cm in the west coast of South Africa. In my study, contributions of cephalopods to the diets of both hake were very low, not exceeding 3 % in many of the length classes. *Todarodes eblanae* had modal contributions of more than 3 % to the length classes of 30-39 cm for *M. capensis* and 20-29 cm for *M. paradoxus*. Increased cannibalism has mostly been reported in larger hake (>60 cm total length) (Macpherson and Gordoa 1994), although cannibalism by *M. capensis* was observed in small (10-20 cm) hake (Pillar and Wilkinson 1995). Small hake (<15 cm), *M. paradoxus* (<40 cm) and *M. capensis* (<40 cm) all had modal contributions of around 1% at all length classes for both hake species, with their upper level CI around 9%.

While the model provided a time-integrated view of the contributions of different prey to the hake diets, the consumption of any prey is also dependent on the prey biomass available to hake. The biomass of the prey used in the model were not determined, therefore a prey biomass survey will help to make inferences on how the prey affects the dynamic of the hake diets and population. The prey biomass survey results could also then be used with isotope mixing models to make inferences on the importance of particular prey to the diet of hake.

The model used (SIAR) allowed for robust dietary solutions of hake species to be derived as the consumer mixture and putative prey items as sources (Parnell *et al.* 2010). Although I am confident in my findings because of the putative nature of the prey used, an increase in sample size of prey would help increase the precision of the model output. Isotope mixing model like SIAR have a number of important limitations as they function on the assumption that all sources are equally available, source parameters are known, trophic fractionation or discrimination is estimated without errors, and that isotope ratios are uncorrelated and they do not allow the diet proportion vector to depend on covariates, such as time (Parnell *et al.* 2010; Galva *et al.* 2012).. The findings of mixing models are also limited by the uncertainties associated with the fractionation factors which are assumed and the isotopic turnover rates which are not known for both hake species. Future research should therefore focus on determining the species-specific fractionation factors and isotopic turnover rates for the two hake species and their major prey. Accurate use of fractionation factors and turnover rates are crucial for quantitative diet estimates from mixing models to help direct future research and improve parameters (i.e. natural mortality) for stock assessment models. Efforts have been made to include as many prey of hake based on published gut content results (Payne *et al.* 1987; Roel and Macpherson 1988; Traut 1996) in the model. The inclusion of many hake prey was done to reduce the effect of missing source biases (Parnell et al. 2013); however because of the compositional nature the models results dietary contribution estimates were limited by the constant sum constraint (Aitchison 1986; Kucera and Malmgren 1998). Future modelling studies are needed to explore ways in which the feasible contributions of different sources are allowed to vary independently in the models. The application of additional independent techniques to assess hake diets such as fatty acids (Chapter 5) will also further refine our understanding of the feeding dynamics of hake species.

CHAPTER 5: DIETARY RELATIONSHIPS OF CAPE HAKE (*MERLUCCIUS CAPENSIS* AND *MERLUCCIUS PARADOXUS*) OFF THE COAST OF NAMIBIA: FATTY ACID SIGNATURE APPROACH

5.1 Introduction

Alternative approaches for detecting dietary relationships among marine organisms like DNA, protein etc., have been explored by ecologists (Blaber and Bulman 1987; Garrison and Link 2000a; Robbins *et al.* 2005; Casper *et al.* 2007; Barnett *et al.* 2010). Fatty acid (FA) compositions are widely used as biochemical dietary tracers in different aquatic ecosystems (Graeve *et al.* 1994; Saito *et al.* 1999; Dalsgaard *et al.* 2003; Robin *et al.* 2003; Arnould *et al.* 2005; El-Sabaawi *et al.* 2009; Haubert *et al.* 2011). FAs are needed by fish as an energy source for growth and reproduction (Fraser and Sargent 1989; Parrish 2012). They have been used to study plankton trophodynamics (Rossi *et al.* 2006; Richoux and Froneman 2009); to distinguish differences in the diets of the sea lamprey (Lança *et al.* 2011); to link the utilization of plankton biomass to enhanced condition of juvenile cod (St John and Lund 1996); to examine the degree of interspecific and intraspecific variability in FA profiles among prey of fish and macro-invertebrates (Czesny *et al.* 2011); and to understand the foraging patterns in cod (*Gadus morhua;*(Kirsch *et al.* 1998).

The basis of the method is that the primary producers have distinctive FAs that can be accumulated in consumer tissues (Kirsch *et al.* 2000; Dalsgaard *et al.* 2003; Brett *et al.* 2006). FAs from primary producers can be incorporated into neutral lipids of consumers with few modifications (Dalsgaard *et al.* 2003). Some FA profiles are specific to certain prey groups (Dalsgaard *et al.* 2003) and can be used to investigate their influences on predator diets (Brett *et al.* 2006; Pethybridge *et al.* 2011). Neutral lipids in higher consumers reflect prey sources more accurately than polar lipids (Jezyk and Penicnak 1966; Parrish *et al.* 1995; Ruess *et al.* 2004). Total FAs of prey can be compared to neutral FAs of predators, as most predators consume their prey whole (Kirsch *et al.* 1998; Dalsgaard *et al.* 2003). However, consumers can selectively metabolize some FAs, therefore FAs can only be used as qualitative and semi quantitative trophic biomarkers (Dalsgaard *et al.* 2003).

The diets of hake (*M. capensis* and *M. paradoxus)* have been studied using stomach content analyses (Payne *et al.* 1987; Roel and Macpherson 1988). Although this method provided important information on hake diets, it has unavoidable biases (See Chapter 1). Biomarkers like FAs which get passed on from food sources to the predator tissues present an alternative approach (Dalsgaard *et al.* 2003; Schmidt *et al.* 2006). Previous studies using lipids (total lipid) indicated that in pre-spawning hake there is a tendency for *M. capensis* to store fats of higher unsaturation than *M. paradoxus* (Wessels and Spark 1973). At the larval stage, hake total FA compositions changed with the developmental stage (Grote *et al.* 2011).

In this study, I contribute to the research efforts of understanding the dietary relationships of *M. capensis* and *M. paradoxus* using time-integrating trophic data. I assessed dietary variations (inter- and intra-specific) of hake and their associations with potential prey using FA profiles. I hypothesized that 1) the two hake species feed on similar prey so they have indistinct FA profiles, 2) there are temporal differences in the diets of the two hake species because of natural variations in prey availability, and 3) the total FA signatures of hake prey are reflected in the neutral FA compositions of the hake. The hypothesis was assessed irrespective of the size of the predators (hake).

5.2 Methods and Materials

5.2.1 Sample collection

Hake samples were collected during a hake survey (January 2011) and a monkfish survey (December 2011), as described in Chapter 2, section 2.1. Potential prey were collected in December 2011 during a monkfish survey as described in Chapter 2, section 2.1.

5.2.2 Laboratory Analysis

Fatty acid analyses were performed as described in Chapter 2, section 2.2.2

5.2.3 Statistical analysis

Fatty acid data were expressed as percentages of the total fatty acids (% TFA). To evaluate the species differences and/or similarities, non-parametric and parametric multivariate analyses were conducted on FA data of hake and some potential prey. A non-parametric multi-dimensional scaling (nMDS) ordination (Euclidean distance) was used to examine the relative similarities of hake neutral FA and to identify if the total FA profiles in potential prey resemble the neutral FA profiles of the two hake species. The stress value was used as a measure of model goodness-of-fit to data. One-way analysis of similarity (ANOSIM) based on Bray-Curtis distances (Clarke and Green 1988; Clarke 1993) was used to test differences in FA profiles among the species and between collection periods (December and January). ANOSIM's R-values were used to indicate the level of similarity, while the p-values were used to estimate the probability to which the test statistics were generated by random chance. Where a significant difference was detected, the contributions of individual FAs to dissimilarities between hake species were assessed using similarity percentage (SIMPER) analysis. To include less common FA that may be informative as trophic biomarkers in the multivariate analyses, the non-parametric methods (nMDS, ANOSIM and SIMPER) used data of all detected FAs (untransformed). Non-parametric multivariate analyses were done using PAST software, version 2.17 (Hammer *et al.* 2001).

Parametric multivariate analyses were conducted using most abundant FAs to minimize effects of variations from minor FAs and to reduce variations that might be artefacts of laboratory procedures. Multivariate analysis of variance (MANOVA) was used to assess if the two hake species had similar neutral FA compositions, and to examine the temporal differences of hake neutral FA compositions. Significance of MANOVA was evaluated with Wilk's λ. MANOVA identifies the differences in FA compositions, but does not reveal which FA contributed most to the observed differences. Discriminant analysis (DA) was used to identify which FAs contributed most to the observed differences. The predictive ability of identified FAs was further tested with DA by assessing success rate of its group classification.

The parametric multivariate methods (MANOVA and DA) require that number of samples must exceed the number of variables to offer some assurance that the covariance matrices are homogeneous (Stevens 1986). FAs were therefore limited to the eleven (Table 5.1) that were detected in amounts >1 % in at least one hake species, and during both sampling periods. Data transformations were done to meet the required multivariate normality. Normalization was performed by centered log-ratio transformation (Aitchison 1986) to avoid the constant sum constraint and to allow individual FAs to vary independently (Kucera and Malmgren 1998). The centered log-ratio transformation was done according to the following equation: $x_y^i =$

ln $\left(\frac{x_{ij}}{g_i}\right)$ (Aitchison 1986), where x_y^i= transformed sample,x_{ij}= proportion of the j^{th} fatty acid in the i^{th}sample and g_i is the geometric mean. Prior to transformation a rounding error (0.05 %) procedure was used (Aitchison 1982) for all the zeros in the data set. The value of zero was fixed to 0.05% for each FA to allow for centered log-ratio transformation (Fry *et al.* 2000). Parametric multivariate analyses were carried out with SPSS (version 20, SPSS Inc., Chicago, IL, USA).

5.3 Results

5.3.1 Hake neutral FA profiles

A total of 110 samples of hake (*M. capensis* and *M. paradoxus)* was analysed for neutral FA composition. Thirty-four FAs, ranging from 14 to 24 carbon atoms in length, were identified from the samples (Appendix 1**)**. Eleven FAs were present in amounts of more than 1 % in at least one species and both sampling periods (Table 5.1). The two species had more polyunsaturated fatty acids (PUFA) than monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) (Fig.5.1). SFAs were dominated by palmitic acid (16:0), MUFAs were dominated by oleic acid (18:1(n-9)), while PUFAs where dominated by docosahexaenoic acid (DHA, 22:6(n-3)), for both species and collection periods.

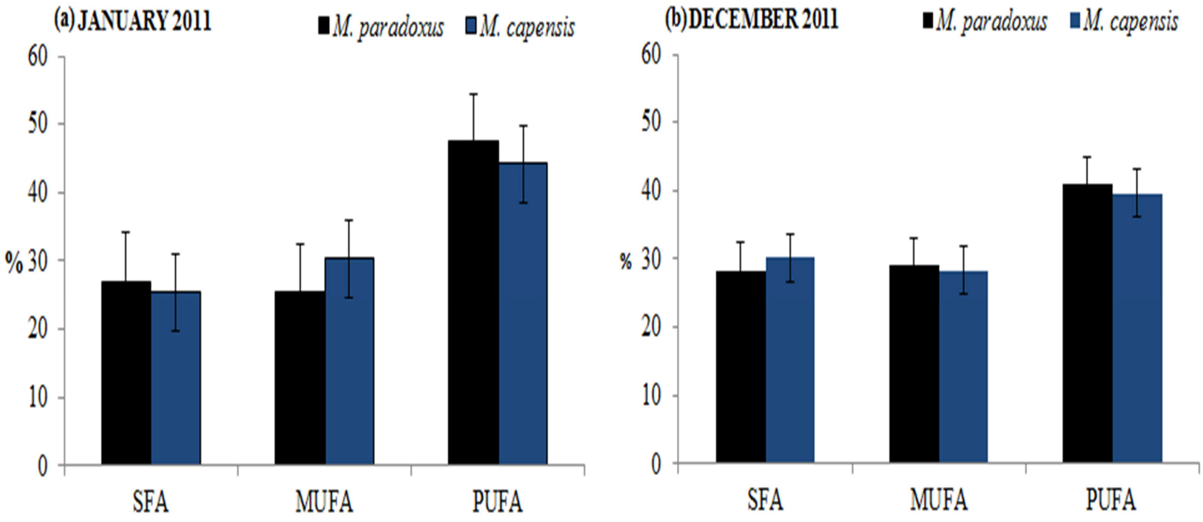

Figure 5.1: Average saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) composition of the two hake species during (a) January 2011, and b) December 2011, with standard error bars.

5.3.2 Hake neutral FA interspecific and intraspecific variations

5.3.2.1 Non-parametric multivariate analyses results

The n-MDS plot (Fig. 5.2) showed little overlap between *M. paradoxus* and *M. capensis* during January 2011(stress = 0.05), while there was a clear separation between them during December 2011 (Fig 5.3, stress = 0.12). ANOSIM indicated that *M. capensis* was not significantly different from *M. paradoxus* in January 2011 (R=0.05, p=0.17). There was a significant difference between *Merluccius capensis* and *M. paradoxus* collected in December 2011(R=0.10, p<0.001). SIMPER analysis showed that three FAs (in decreasing order of importance: 22:6(n-3), 18:1(n-9) and 20:5(n-3)) explained 52 % of the dissimilarities in December 2011 (Table 5.1). *Merluccius capensis* collected in January 2011 was significantly different from that collected in December 2011(R= 0.9, p < 0.001), with 22:6(n-3), 16:0 and 18:1(n-9) being the dominant contributors to the differences (SIMPER, Table 5.2). *Merluccius paradoxus* collected in January 2011 was also significantly different from those collected in December 2011(R= 0.91, p<0.001), and three FAs (in decreasing order of importance: 22:6(n-3), 16:0 and 18:1(n-9)) were the dominant contributors to the differences (SIMPER, Table 5.3).

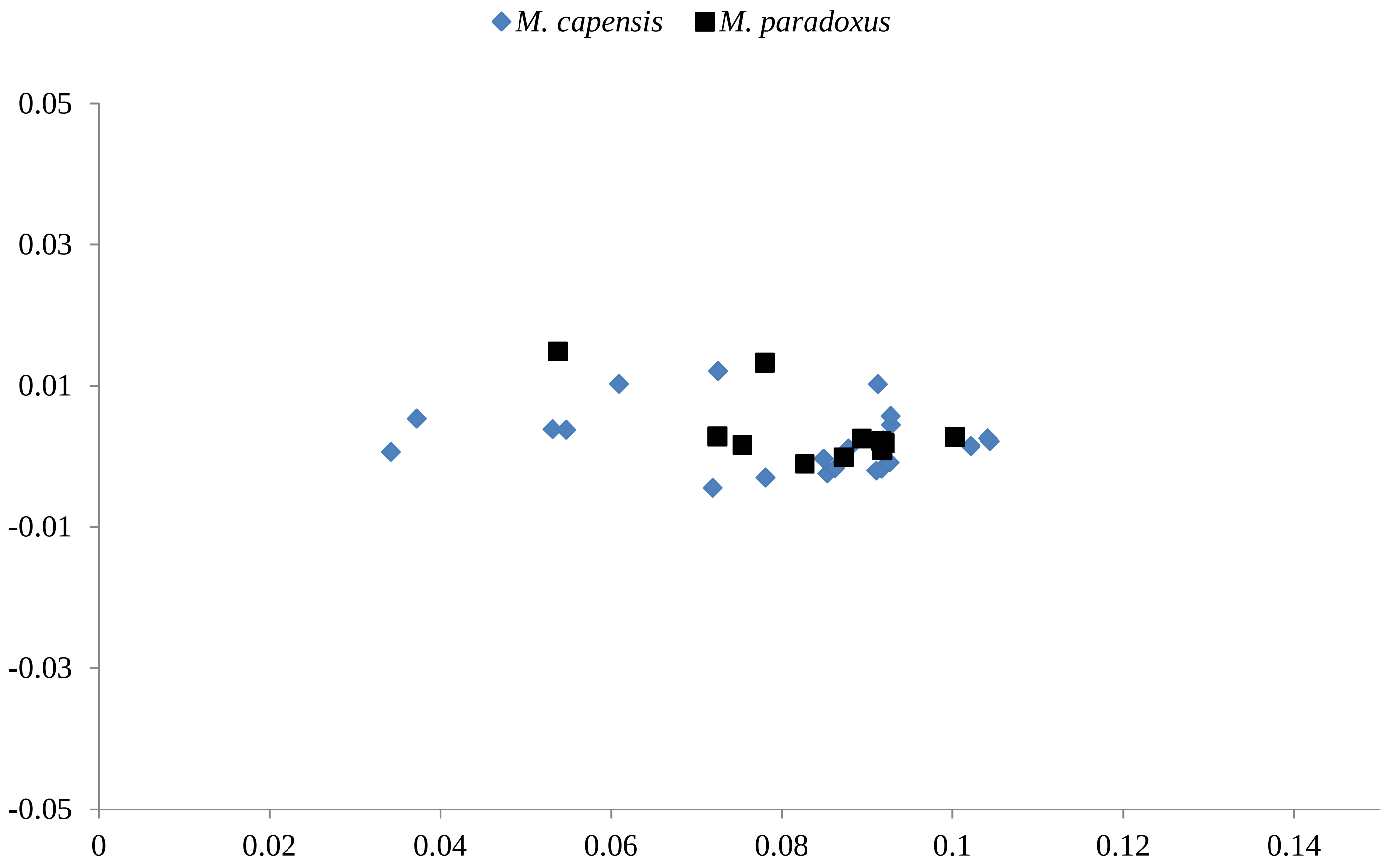

Figure 5.2: nMDS output of hake neutral fatty acids collected during December 2011 (Stress = 0.05)

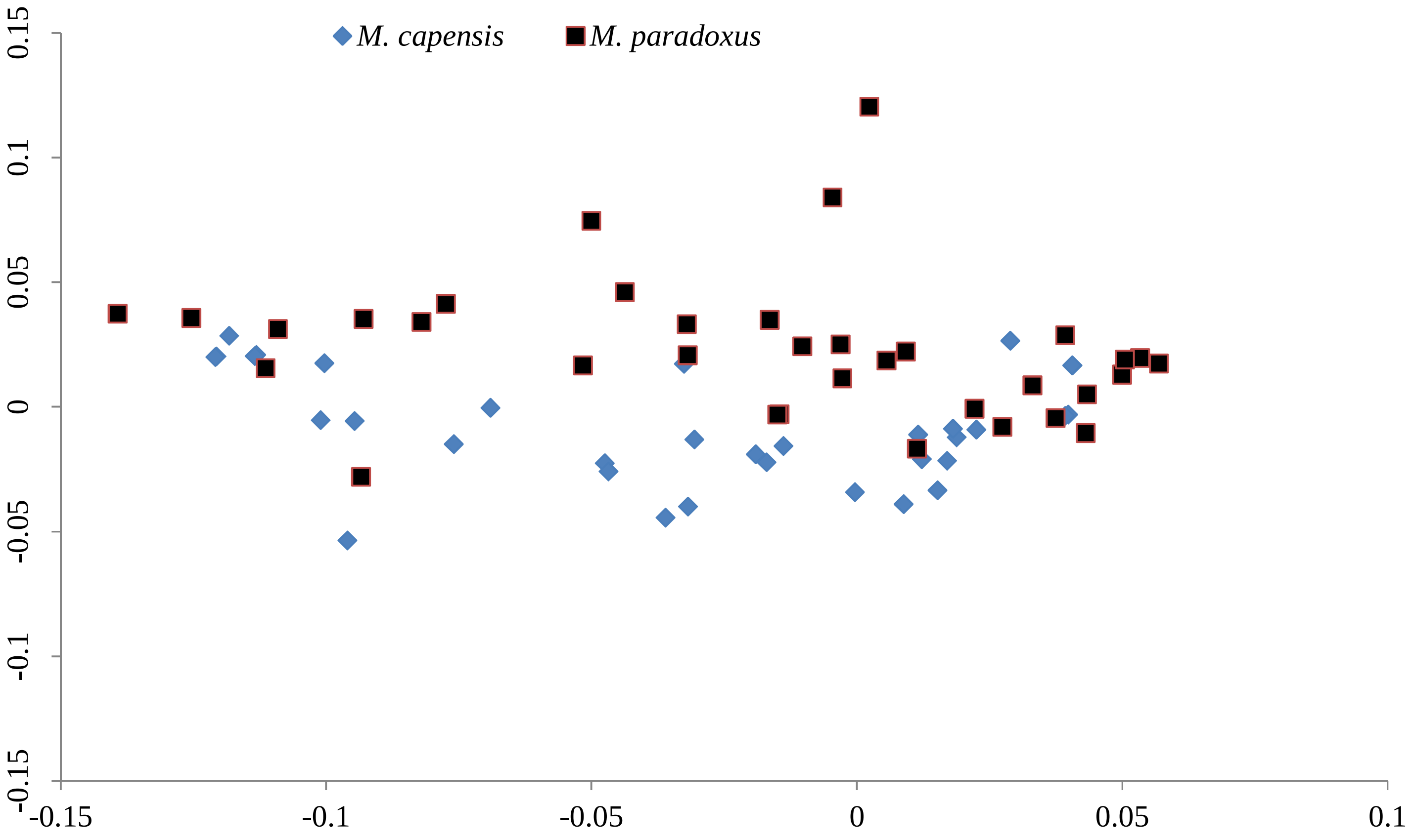

Figure 5.3: nMDS output of hake neutral fatty acids collected during January 2011 (stress = 0.12)

Table 5.1: SIMPER summary of fatty acids accounting for 90% of the dissimilarity between the tissues of *M. capensis* and *M. paradoxus* collected in December 2011.Values are average dissimilarities (Av.dissim), contributions to the dissimilarities (contr.%), cumulative contributions to the dissimilarities (cumulative %) and their abundances in the two hake species (proportions %).

				Proportion (%)	
FA	**Av. dissim**	**Contrib. %**	**Cumulative %**	***M. capensis***	***M. paradoxus***
22:6(n-3)	4.777	23.44	23.44	22.2	27.8
18:1(n-9)	3.582	17.58	41.02	14.7	17.6
20:5(n-3)	2.399	11.77	52.79	12.5	9.81
16:1(n-7)	1.298	6.368	59.16	2.59	1.34
16:0	1.286	6.308	65.47	20.2	20.4
20:1(n-9)	1.253	6.147	71.62	4.45	4.89
22:1(n-9)	1.078	5.29	76.91	2.94	2.47
18:0	1.049	5.145	82.05	6.26	5.24
18:1(n-7)	0.6182	3.034	85.09	3.56	2.73
14:0	0.5554	2.725	87.81	2.23	1.45
20:4(n-6)	0.4312	2.116	89.93	2.21	1.94

Table 5.2: SIMPER summary of fatty acids accounting for 90% of the dissimilarity of *M. capensis* tissues collected between January 2011 and December 2011. Values are average dissimilarities (Av.dissim), contributions to the dissimilarities (contr. %), cumulative contributions to the dissimilarities (cumulative %) and their abundance in *M. capensis* during the two sampling periods (proportions %).

				Proportion (%)	
FA	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Jan 2011**	**Dec 2011**
22:6(n-3)	18.26	21.42	21.42	2.58	22.2
16:0	17.17	20.14	41.56	1.72	20.2
18:1(n-9)	12.5	14.66	56.22	1.26	14.7
20:5(n-3)	10.6	12.43	68.65	1.1	12.5
18:0	5.399	6.333	74.99	0.441	6.26
20:1(n-9)	3.858	4.525	79.51	0.3	4.45
18:1(n-7)	3.017	3.539	83.05	0.311	3.56
22:1(n-9)	2.699	3.166	86.22	0.0216	2.94
16:1(n-7)	2.294	2.691	88.91	0.194	2.59
14:0	1.982	2.325	91.23	0.0941	2.23

Table 5.3: Summary of fatty acids accounting for 90% of the dissimilarity of *M. paradoxus* tissues collected between January 2011 and December 2011. Values are average dissimilarities (Av.dissim), contributions to the dissimilarities (contr.%), cumulative contributions to the dissimilarities (cumulative %) and their abundance in *M. paradoxus* during the two sampling periods (proportions %).

				Proportion (%)	
FA	**Av. dissim**	**Contrib. %**	**Cumulative %**	**Jan2011**	**Dec2011**
22:6(n-3)	21.37	26.51	26.51	5.4	27.8
16:0	16.09	19.96	46.47	7.2	20.4
18:1(n-9)	14.33	17.78	64.25	7.9	17.6
20:5(n-3)	7.733	9.594	73.85	3.36	9.81
18:0	4.145	5.143	78.99	1.71	5.24
20:1(n-9)	3.942	4.89	83.88	1.96	4.89
18:1(n-7)	2.293	2.845	86.72	1.64	2.73
22:1(n-9)	1.983	2.46	89.19	0.863	2.47
20:4(n-6)	1.545	1.917	91.1	0.772	1.94
22:6(n-3)	21.37	26.51	26.51	5.4	27.8
16:0	16.09	19.96	46.47	7.2	20.4

5.3.2.2 Parametric multivariate analyses results

Eleven FAs (Table 5.1), that were present in amounts greater than 1 % in at least one hake species and in both sampling periods, were used in the parametric multivariate analyses. When all the samples from both sampling periods were combined per species, MANOVA indicated that there was a significant difference between M. *capensis* and *M. paradoxus* neutral FA compositions (Wilk's λ = 0.687, p<0.001). The DA indicated that 22:6(n-3) (Wilk's λ=0.95, p=0.02), 22:5(n-3) (Wilk's λ=0.88, P=0.001), 20:1(n-9) (Wilk's λ=.0.819, p<0.001), and 16:1(n-7) (Wilk's λ=0.790, p<0.001) yielded the maximum discrimination of the two species. DA classified them with 69.5 % success rate. Samples were further separated between the January 2011 and December 2011 collections.

For January 2011, MANOVA indicated that there was a significant difference between neutral FA compositions of *M. capensis* and *M. paradoxus* (Wilk's λ== 0.464, p<0.001). The DA indicated that 22:1(n-9) (Wilk's λ=0.737, p=0.001), and 18:0 (Wilk's λ=0.624, p<0.001) separated the two species. The DA correctly classified 80.5% of samples as *M. capensis* and *M. paradoxus.*

During the December 2011 sampling period**,** MANOVA also indicated significant differences between neutral FA composition of *M. capensis* and *M. paradoxus* (Wilk's λ = 0.578, p<0.001). The DA indicated that 22:5(n-3) (Wilk's λ=0.877, p=0.003), 22:6(n-3) (Wilk's λ= 0.792, p<0.001), and 20:1(n-9) (Wilk's λ=0.725, p<0.001) yielded the maximum separation of the two species. DA correctly classified 78.6% of original grouped samples.

There was a significant difference between *M. capensis* collected in January 2011 and December 2011(MANOVA, Wilk's λ=0.49, p<0.001). DA indicated that 18:0 (Wilk's λ=0.108, p<0.001), 2:1(n-9) (Wilk's λ=0.06, p<0.001) and 16:0 (Wilk's λ=0.05, p<0.001) differentiated M. *capensis* samples. The DA correctly classified 100% of samples into January 2011 and December 2011 collections periods. There was also significant difference in *M. paradoxus* neutral FA composition between the two collection periods (Wilk's λ = 0.21, p<0.001). DA results indicated that only 22:6(n-3) (Wilk's λ = 0.27, p<0.001) yielded maximum differentiations of *M. paradoxus* from the two sampling periods. The model classified 98% of the samples into January 2011 and December 2011 collection periods.

Table 5.4: Fatty acid (FA) compositions (mean%± standard deviation) of the two hake species (*M. capensis*, *M. paradoxus*) during the two collection periods. FAs that were present in amounts less than 1 % in one species or not present in both sampling periods are not shown (see Appendix 1 for the full list)

		January 2011		**December 2011**	
		M. capensis	***M. paradoxus***	***M. capensis***	***M. paradoxus***
		n = 25	*n* = 15	*n* = 34	*n* = 36
Length range		16-57 cm	22-59 cm	24-69 cm	27-54 cm
FA	**Common name**				
16:0	palmitic acid	19.44 ± 3.72	18.69 ± 2.85	20.21±1.61	20.44±3.47
16:1(n-7)	palmitoleic acid	2.18 ± 1.22	2.25 ± 1.40	2.59±2.63	1.34±1.91
18:0	stearic acid	5.51 ± 1.39	4.54 ± 1.26	6.26±2.07	5.23±1.68
18:1(n-9)	oleic acid	12.26 ± 7.49	14.83 ± 5.95	14.70±5.11	17.57±6.84
18:1(n-7)	vaccenic acid	3.47± 1.06	3.68 ± 0.85	3.55±0.93	2.73±1.23
20:1(n-9)	eicosenoic acid	2.63 ± 2.51	4.04 ± 2.48	4.45±2.09	4.89±2.35
20:4(n-6)	arachidonic acid (AA)	2.42 ± 0.80	2.15 ± 0.47	2.21±0.92	1.94±0.62
20:5(n-3)	eicosapentaenoic acid (EPA)	13.21±7.16	8.85 ± 3.00	12.52±3.66	9.81±4.02
22:1(n-9)	erucic acid	0.19 ±0.54	1.49 ± 1.70	2.94±2.14	2.46±1.69
22:5(n-3)	docosapentaenoic acid (DPA)	1.71 ± 0.76	1.27 ± 0.73	1.88±0.74	1.27±0.56
22:6(n-3)	docosahexaenoic acid (DHA)	27.99 ± 9.54	27.92±11.59	22.17±6.83	27.81±7.92

5.3.3 Prey total FA profiles

A total of 58 samples of potential prey of hake was analysed for TFA composition (Table 5.4). The potential prey used were lantern belly (*Synagrops microlepis),* shortnose greeneye *(Chlorophthalmus agassizi),* cape anchovy *(Engraulis capensis),* pencil cardinal *(Epigonus denticulatus),* blackbelly rosefish *(Helicolenus dactylopterus)*, flying squid *(Todarodes sagittatus)*, smalltooth grenadiers *(Nezumia micronychodon),* cape dragnet *(Paracallionymus costatus),* and pelagic goby (*Sufflogobius. bibartus).* Thirty-four FAs, ranging from 14 to 22 carbon atoms in length, were identified from the prey (Table 5.4). Most prey had more PUFA than MUFA and SFA, only lantern belly (*S. microlepis*) had more MUFA than both PUFA and SFA (Fig. 2). SFA were dominated by palmitic acid (16:0), MUFAs were dominated by oleic acid (18:1(n-9)) and PUFAs were dominated by docosahexaenoic acid (DHA, 22:6(n-3) in all prey.

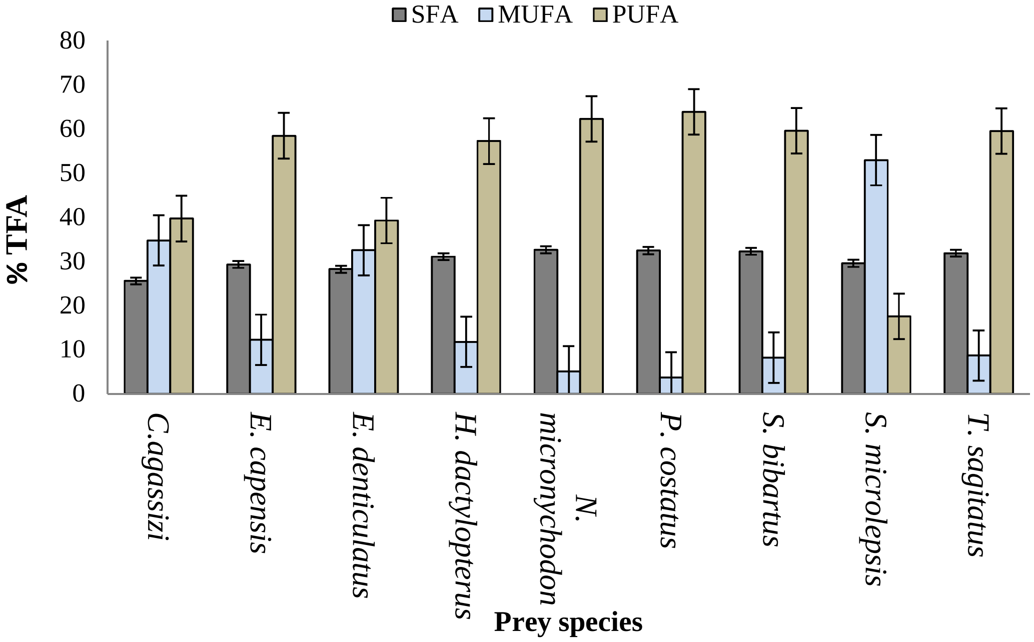

Figure 5.4: Average saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) compositions of prey species, with standard error bars.

Table 5.5: Fatty acid compositions (mean% ± standard deviation) of potential prey of hake collected in December 2011.

	S .microlepis (n=3)	***C. agassizi (n=9)***	***E. capensis (n=14)***	***E. denticulatus (n=6)***	***H. dactylopterus (n=12)***	***T. sagittatus (n=1)***	***N. micronychodon (n=6)***	***P. costatus (n=4)***	***S. bibartus (n=5)***
14:0	7.84±0.83	5.14±2.45	3.15±1.83	2.82±2.18	1.26±0.66	1.30	0.97±0.11	1.12±0.23	1.52±0.32
i-15:0	0.13±0.01	0.124±0.07	0.06±0.07	0.50±0.78	0.07±0.06	0.03	0.07±0.07	0.06±0.07	0.08±0.08
15:0	0.29±0.03	0.314±0.13	0.33±0.22	0.47±0.22	0.43±0.18	0.44	0.49±0.23	0.67±0.14	0.61±0.17
16:0	15.60±13.53	15.29±11.50	19.20±12.80	15.25±10.43	21.74±2.35	25.14	21.79±1.39	20.92±2.06	17.98±2.97
16:1(n-7)	1.80±2.71	0.48±1.17	2.62±7.51	0.17±0.19	0.33±0.66	0	0.12±0.18	0.11±0.22	0.84±1.31
i-17:0	0.30±0.06	0.30±0.15	0.18±0.16	0.65±0.33	0.42±0.35	0.12	0.36±0.46	0.76±0.07	0.20±0.31
ai-17:0	0.09±0.01	0.09±0.06	0.08±0.09	0.31±0.22	0.16±0.26	0	0.21±0.33	0.18±0.22	0.29±0.33
17:0	0.48±0.06	0.62±0.18	0.93±0.28	0.73±0.21	0.63±0.27	0.53	0.80±0.52	1.21±0.32	1.06±0.56
17:1(n-7)	0.38±0.09	0.39±0.27	0.18±0.22	0.46±0.22	0.40±0.23	0.08	0.29±0.25	0.48±0.79	0.18±0.20
18:0	4.14±3.59	3.22±2.24	5.22±2.41	3.77±2.66	5.90±2.26	4.29	7.74±1.22	7.01±0.93	10.27±2.62
18:1(n-9)	25.92±15.08	15.07±10.37	4.52±2.58	12.34±12.06	7.59±6.23	1.21	3.68±1.36	2.75±0.10	5.76±0.65
18:1(n-7)	3.19±0.25	11.20±14.51	1.64±2.96	11.23±16.13	0.80±1.45	0	0.00±0.00	0.00±0.00	0.04±0.10
18:1(n-5)	13.68±23.41	0.69±1.23	0.29±0.94	0.73±1.56	0.06±0.11	0	0.07±0.17	0.10±0.12	0.05±0.12
18:2(n-6)	0.00±000	0.18±0.36	0.33±0.64	0.14±0.37	0.32±0.50	0	0.23±0.57	0.00±0.00	0.56±0.81
18:3(n-6)	0.00±000	0.02±0.03	0.01±0.02	1.53±3.94	0.00±0.00	0	0.00±0.00	0.00±0.00	0.00±0.00
18:3(n-3)	0.00±000	0.01±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0	0.00±0.00	0.00±0.00	0.00±0.00

Table 5.5 continued

18:4(n-3)	0.46±0.06	0.40±0.42	0.26±0.36	0.19±0.18	0.00±0.00	0	0.00±0.00	0.09±0.17	0.23±0.34
20:0	0.37±0.05	0.25±0.13	0.08±0.11	0.28±0.29	0.30±0.65	0	0.03±0.08	0.42±0.59	0.24±0.20
20:1(n-9)	4.70±4.15	3.27±2.93	1.56±0.77	5.54±7.57	1.93±1.92	6.83	0.95±0.84	0.25±0.29	0.98±0.85
20:2(n-6)	0.18±0.02	0.20±0.10	0.14±0.16	0.39±0.41	0.09±0.19	0.21	0.08±0.20	1.28±2.56	0.09±0.21
20:3(n-6)	0.01±0.02	0.03±0.06	0.07±0.13	0.16±0.28	0.08±0.23	0	0.13±0.32	0.00±0.00	0.00±0.00
20:4(n-6)	0.63±0.08	1.73±1.08	1.68±1.38	3.27±2.46	4.10±1.53	1.40	4.63±0.96	4.12±2.75	6.42±2.09
21:0	0.08±0.09	0.08±0.11	0.05±0.11	3.28±8.47	0.02±0.06	0	0.05±0.13	0.05±0.10	0.00±0.00
20:3(n-3)	0.04±0.06	0.08±0.08	0.06±0.09	0.2±0.26	0.07±0.13	0	0.23±0.41	0.08±0.16	0.00±0.00
20:4(n-3)	0.57±0.06	0.55±0.24	0.18±0.19	0.59±0.91	0.18±0.41	0	0.00±0.00	0.00±0.00	0.18±0.28
20:5(n-3)	5.53±0.59	9.01±2.51	9.71±3.51	8.47±4.57	8.25±2.76	13.04	10.18±2.67	11.33±1.40	15.90±6.54
22:0	0.24±0.03	0.10±0.10	0.02±0.04	0.14±0.13	0.12±0.28	0	0.10±0.24	0.06±0.11	0.00±0.00
22:1(n-9)	3.26±0.37	3.64±2.24	1.42±0.95	2.05±2.58	0.64±0.90	0.54	0.00±0.00	0.00±0.00	0.34±0.32
21:5(n-3)	0.00±000	0.00±000	0.10±0.39	0.00±0.00	0.00±0.00	0	0.00±0.00	0.88±1.76	0.00±0.00
22:4(n-6)	0.04±0.06	0.17±0.12	0.09±0.33	0.42±0.40	0.48±0.47	0	0.22±0.53	0.42±0.49	0.68±0.62
22:4(n-3)	0.00±000	0.10±0.29	0.00±0.00	0.00±0.00	0.00±0.00	0	0.00±0.00	0.00±0.00	0.00±0.00
22:5(n-6)	0.18±0.02	0.44±0.30	0.23±0.28	0.75±0.67	1.24±0.56	0.34	0.96±0.81	0.44±0.50	0.80±0.83
22:5(n-3)	1.63±0.18	2.35±0.95	1.10±1.00	3.51±2.81	3.29±1.43	0.69	3.20±1.75	1.98±1.48	3.72±0.55
22:6(n-3)	8.25±1.00	24.44±14.31	44.48±11.28	19.65±15.32	39.10±7.37	43.83	42.41±4.71	43.25±2.56	30.97±7.81

5.3.4 Hake and prey FA associations

The nMDS plot of hake samples collected in January 2011 showed all hake clustered together, with a clear separation from the potential prey that were collected in December 2011 (Fig. 5.5). The nMDS plot of hake collected in December 2011 showed two clusters of potential prey species. Lantern belly (S. *microlepis),* shortnose greeneye *(C. agassizi) and* pencil cardinal *(E. denticulatus)* were positioned close to both *M. capensis* and *M. paradoxus*. Cape anchovy *(E. capensis),* blackbelly rosefish *(H. dactylopterus),* flying squid *(T. sagittatus),* smalltooth grenadiers *(N. micronychodon),* cape dragnet *(P. costatus),* and pelagic goby (*S. bibartus)* were more similar to *M. paradoxus* than to *M. capensis* (Fig. 5.6).

The ANOSIM results indicated that TFA compositions of most prey were significantly different from hake neutral FA compositions (Table 5.5, R = 0.51, p<0.001). Flying squid (*T. sagittatus*) were not significantly different from *M. paradoxus* collected in December 2011 (Table 5.5, R=0.51, p=0.11).

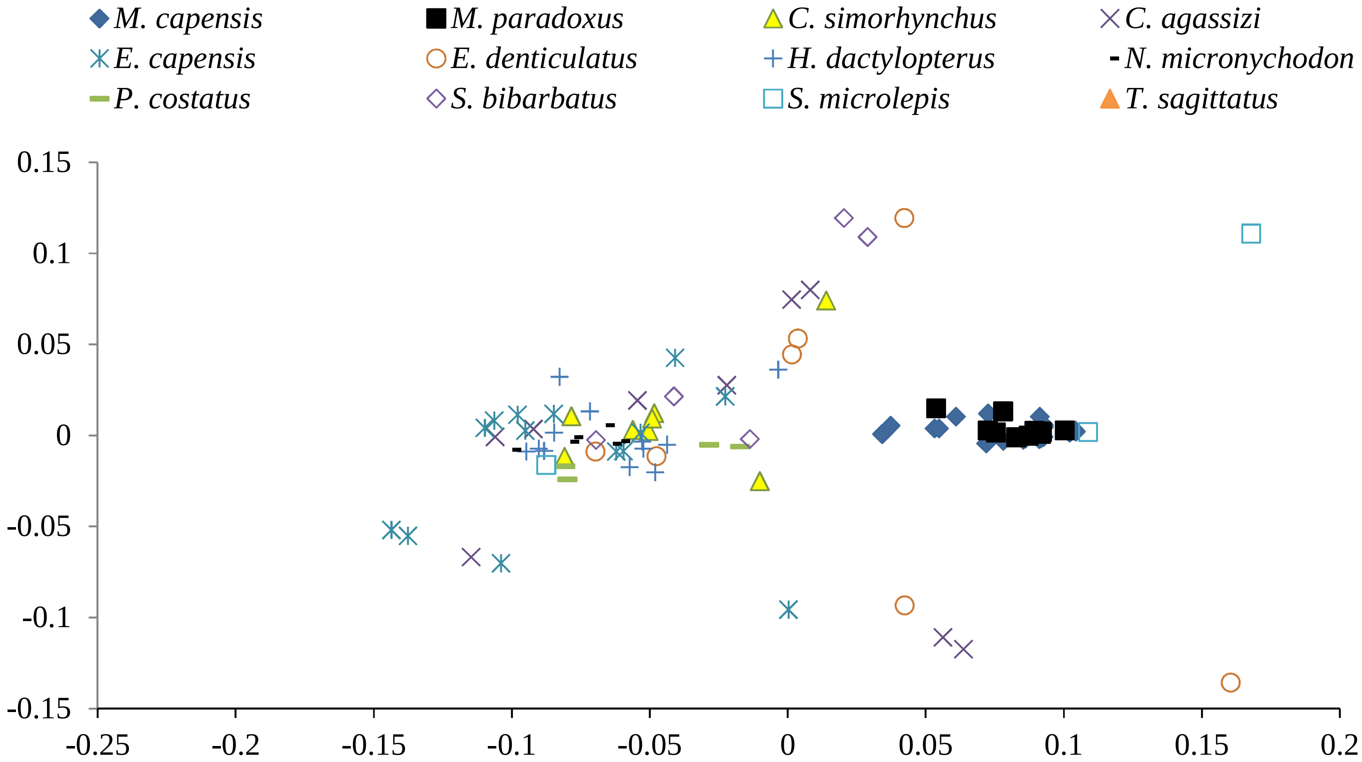

Figure 5.5: nMDS output of hake neutral fatty acids collected during January 2011 and total fatty acids of prey collected in December 2011(stress = 0.09).

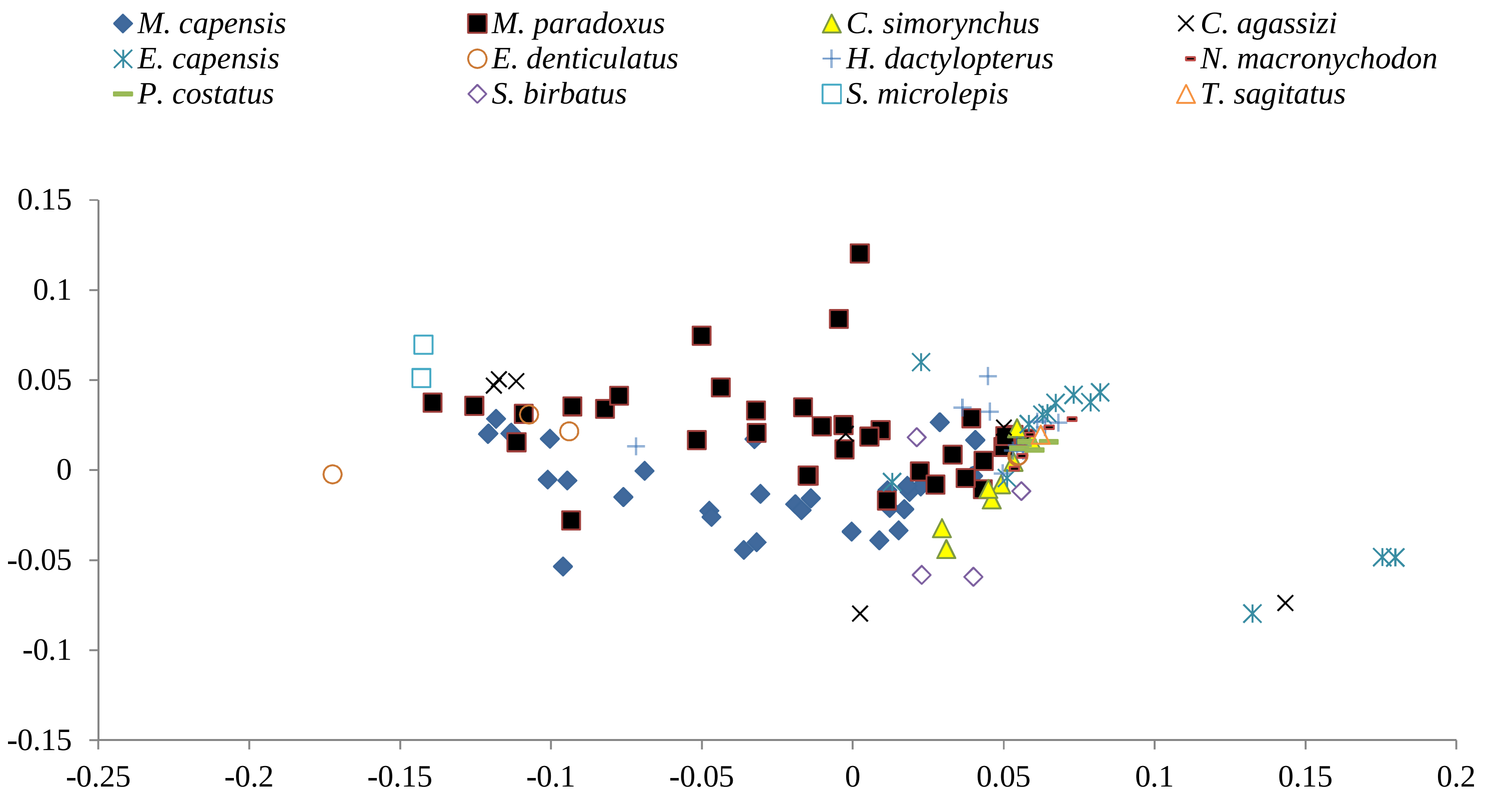

Figure 5.6: nMDS output of hake neutral fatty acids collected in December 2011and total fatty acids of prey collected in December 2011 (stress = 0.20).

Table 5.6: Pairwise comparisons of total fatty acids in prey and neutral fatty acids in hake (ANOSIM, R=0.51). Circled values indicate significant pairwise ANOSIM results ($p > 0.05$).

Prey species	***M. capensis***		***M. paradoxus***	
	Jan 2011	**Dec 2011**	**Jan 2011**	**Dec 2011**
C. simorhynchus	0.0001	0.0001	0.0003	0.0001
C. agassizi	0.0001	0.0001	0.0002	0.0002
E. capensis	0.0001	0.0001	0.0001	0.0001
E. denticulatus	0.0001	0.0002	0.0002	0.0003
H. dactylopterus	0.0001	0.0001	0.0001	0.0001
N. micronychodon	0.0001	0.0001	0.0004	0.0001
P. costatus	0.0001	0.0001	0.0054	0.0003
S. bibartus	0.0001	0.0001	0.002	0.0003
S. microlepsis	0.0004	0.0003	0.022	0.0006
T. sagittatus	0.0378	0.0285	0.0003	0.107*

5.4 Discussion

FA signatures from the two hake species *(M. paradoxus* and *M. capensis)* were significantly different, with a significant temporal change in hake neutral FA compositions observed between the two sampling periods. The comparison of hake neutral FAs and TFAs of potential prey showed their relative associations, indicating prey influences on *M. paradoxus* and *M. capensis* diets. Flying squid (*T. sagittatus*) in particular showed a significant influence on the diet of *M. paradoxus*. My study showed the usefulness of neutral FAs of hake and TFA of prey sources to investigate their trophic relationships.

The FA profiles in the two hake species and prey were similar to those of the most abundant FAs found in marine fish (Budge *et al.* 2002; Guil-Guerrero *et al.* 2011; Pethybridge *et al.* 2011). Hake and its potential prey had more PUFAs than other type of fatty acids except *S. microlepsis,* which had more MUFAs than PUFAs and SFAs. Most marine fish lack delta -5 desaturase activities needed to biosynthesize PUFAs and therefore have an absolute dietary requirement for unsaturated FAs (Tocher and Ghioni 1999; Hastings *et al.* 2001; Nichols 2003)**.** PUFAs are transferred through the food chain from primary producers that carry out elongation of short-chain FAs to form PUFAs (Calder 2001; Nichols 2003). High PUFA content than other FA types in the two species of hake (Fig 5.1) is in agreement with the findings of previous studies of the two hake species (Wessels and Spark 1973; Grote *et al.* 2011). The increased MUFA, as compared to other types of FAs, in *S. microlepsis* (Fig. 5.4), could be because it is has more fat than the other species considered. Fatty fish accumulate depot lipids composed mainly of saturated and monounsaturated FAs (Kozlova and Khotimchenko 2000). Palmitic acid (16:0), which was the most abundant SFA in the hake and prey FA compositions, is possibly related to the influence of phytodetritus in their diets (Reemtsma *et al.* 1990). The high proportions of oleic acid (18:1(n-9)) among the MUFAs (Table 5.4) may reflect the influence of crustaceans in both hake and prey diets (Lee *et al.* 1971; Morris 1971). However, palmitic acid (16:0) and oleic acid (18:1(n-9)) can be synthesized de novo by some animals, hence their abundance in hake and prey may not only reflect dietary influences (Weers and Gulati 1997; Weers *et al.* 1997). For PUFAs, hake and prey showed similar patterns compared with other marine fish, where DHA (22:6(n-3)) is the dominant PUFA (Kozlova and Khotimchenko 2000; Koussoroplis *et al.* 2010; Lança *et al.* 2011; Gladyshev *et al.* 2013). High concentrations of DHA among the PUFAs of hake were observed by Wessels and Spark (1973), and has been found in liver oil of other hake e.g. *Merluccius hubbsi* (Méndez 1997). The level of DHA observed, is likely from the influences of zooplanktivorous hake prey feeding on zooplankton such as copepods, which have high DHA content (Huynh and Kitts 2009; Gladyshev *et al.* 2013). Grote *et al.* (2011) observed the dominance of palmitic acid, oleic acid and DHA in eggs and larvae of the two hake species. It is therefore plausible that the dominance of these FAs can also be attributed to increased metabolic requirements of these FAs by the two hake species (Mayor *et al.* 2013), rather than simply to their abundance in hake diet.

Multivariate tests revealed significant differences/dissimilarities in the FA profiles of the two hake species, indications of the differences of their dietary sources. The differences in hake species FA profiles reflect the influence of dietary items with different FA profiles and possibly the effect of their different metabolic activities (Young *et al.* 2010). The group of FAs that yielded the maximum discrimination between the two species changed between sampling periods. The temporal variations could be a result of seasonal variation in quantity and quality of prey resources that influence FA profiles (Elizabeth 2000; Czesny *et al.* 2011; Braeckman *et al.* 2012; Gonçalves *et al.* 2012; Dethier *et al.* 2013). The temporal variations of *M. capensis* were reflected in stearic acid (18:0), erucic acid 2:1(n-9) and palmitic acid (16:0), and discriminant analysis correctly classified 98 % of the samples into the correct collection period. For *M. paradoxus,* only DHA yielded maximum discrimination between the collection periods (100 % classification success rate), which showed that there was less temporal variation in its FA composition. The decreased temporal variations in *M. paradoxus* tissues can be an indication of dietary specialization or the dominance of similar prey species in their diets during the different collection periods.

The fact that stearic acid (18:0), which can be influenced by changes in temperature (Tanakol *et al.* 1999) was among the FAs that contributed to dissimilarities between the two hake species can be an indication of temperature influence. It is possible that there are differences in metabolic rates of different fish species with changes in temperature (Childress *et al.* 1990), and temperature normally decreases with ocean depth. Although the two hake species have overlapping depth distributions, *M. capensis* is found at shallower depths than *M. paradoxus* (Burmeister 2001). Further studies are needed to look at the influence of other factors (i.e.

temperature) on hake FA composition to better delineate the influence of diet on FAs. A study of dietary lipids and temperature interaction that influenced tissue FA compositions of Atlantic salmon indicated that temperature had a marked influence on polar lipid than neutral lipid compositions (Jobling and Bendiksen 2003).

One of the FAs that consistently discriminated the two hake species irrespective of the sampling period was erucic acid (20:1(n-9)). Erucic acid (20:1(n-9)) content in hake may reflect their consumption of organisms such as crustaceans and squid (Phillips 2003; Beckmann *et al.* 2013). Distinctions between the diets of *M. capensis* and *M. paradoxus* may potentially be based on differences in the relative numbers of crustaceans and cephalopods in their diets. Traut (1996) indicated that crustaceans and cephalopods were of minor importance to *M. capensis,* but contributed relatively more to the diet of *M. paradoxus*. The flying squid (*T. sagittatus*) was the only prey species whose TFA was not significantly different from *M. paradoxus* neutral FAs. Although the above was based on a single marginal p-value (Table 5.6), it can be an indication of an increased influence of cephalopods in the diet of *M. paradoxus* compared with that of *M. capensis*.

The nMDS analyses showed qualitative separations, with little overlap, between the two hake species based on their FA compositions. The overlap could be a result of dietary source overlap, thereby supporting findings from stomach content studies (Roel and Macpherson 1988; Traut 1996). The FA compositional overlap could also result from similar phylogenetic positions of the species, as such taxonomic relationships can lead to similar FA compositions in different species even if they have different feeding strategies (Makhutova *et al.* 2011). The nMDS visualization of potential prey TFA profiles together with hake neutral FA profiles indicated some associations. *Synagrops microlepis, E. denticulatus* and *C. agassizi* had similar TFA to both hake species, which may imply that they have similar influences on hake diets. *Engraulis capensis, H. dactylopterus, T. sagittatus, N. micronychodon, P. costatus* and *S. bibartus* had similar profiles with *M. paradoxus* only. Stable isotope analysis indicated that *M. paradoxus* was more piscivorous in its feeding than *M. capensis* (Iitembu *et al.* 2012). The stable isotope results support the FAs results in that all the prey species that have similar FAs to *M. paradoxus* also occupied higher trophic levels than other prey (Chapters 3 and 4).

This study has shown that FA compositions can be used to differentiate diets between closely related and sympatric aquatic animals. However, it becomes difficult to predict how individual FAs behave at higher trophic levels because of elongation and desaturation processes occurring throughout a food web (Graeve *et al.* 1994; Dalsgaard *et al.* 2003; Dalsgaard and John 2004; Pollierer *et al.* 2010). The FA compositions of consumers are influenced by their diets, but preferential accumulations, modifications, and biosynthesis of some FAs are expected (Hessen and Leu 2006; Guschina and Harwood 2006; Beckmann *et al.* 2013). No single FA can be assigned uniquely to any one species (Dalsgaard *et al.* 2003). To use FAs with confidence in dietary studies, it is important to understand the direct effects of diet on FA compositions and turnover in predator tissues (Kirsch *et al.* 2000). Future research should therefore involve controlled laboratory studies whenever possible so that we may better understand the turnover and deposition of FAs in hake tissues. The utilization of FAs as dietary tracers in laboratory studies can be used to understand processes occurring in trophic food webs, so that we may trace paths of FAs to hake in open-ocean systems. I recommend that researchers incorporate a monthly sample collection to allow for a comprehensive evaluation of the dietary relationships between species.

CHAPTER 6: SOME ASPECTS OF THE TROPHIC RELATIONSHIPS OF HAKE (*MERLUCCIUS CAPENSIS* AND *MERLUCCIUS PARADOXUS*) AND SHARKS *(CENTROPHORUS SQUAMOSUS, DEANIA CALCEA AND D. PROFUNDORUM*) OFF NAMIBIA: A STABLE ISOTOPE APPROACH

6.1 Introduction

Trophic relationships play a major role in the structuring of aquatic systems (Vander Zanden *et al.* 2000; Heithaus *et al.* 2008; Laroche *et al.* 2008). Understanding the trophic relationships among different marine species is necessary towards researchers gaining insight into the roles they play in influencing food web structure (Pethybridge *et al.* 2011). The marine waters off Namibia have very high level of upwelling based primary production that support a very large biomass of marine species, aiding complex trophic linkages (Hutchings *et al.* 2009).

Hake (*M. capensis* and *M. paradoxus*) are important secondary and tertiary consumers in the Benguela Current ecosystem (Roel and Macpherson 1988; Heymans *et al.* 2004). Sharks such as *Centrophorus squamosus* (Bonnaterre, 1788), *Deania calcea* (Lowe, 1839), and *Deania profundorum* (Smith & Radcliffe, 1912) have overlapping depth distributions with hake (Bianchi *et al.* 1999; Burmeister 2001). Sharks are known top predators (Cortés 1999; Rogers *et al.* 2012) that feed on a variety of fish (Ebert 1991, 1994; Barnett *et al.* 2012), pinnipeds and cetaceans (Pethybridge *et al.* 2011). Stomach content analyses of *C. squamosus*, *D. calcea* and *D. profundorum* have indicated that they feed on hake (Yano 1991; Ebert *et al.* 1992; Ebert 1994), and other prey common with hake (Payne *et al.* 1987; Roel and Macpherson 1988**).** The trophic relationships between hake (*M. capensis and M. paradoxus*) and sharks (*C. squamosus, D. calcea* and *D. profundorum)* in Namibian waters are not well understood, and little is known about the impacts these sharks have on hake biomass.

Fisheries management is moving towards an ecosystem-based approach (Roux and Shannon 2004; Cochrane *et al.* 2009) that requires an understanding of species trophic interactions (Shannon *et al.* 2006). This study contributes towards research efforts to aid consideration of trophic dynamics in fisheries management decisions (e.g. total allowable catches), and evaluate the potential impact that sharks have on the hake biomass. Several studies, from other ecosystems other than the Benguela, have used stable isotopes to look at the trophic roles of sharks (Davenport and Bax 2002; Estrada *et al.* 2003; Domi *et al.* 2005).

I compared the isotopic niches of two hake and three shark species, as isotopic niche width correlates positively with diet breadth of species (Cummings *et al.* 2012) and can be used to make inferences on their population trophic niche width (Syväranta et.al 2013). I investigated their predator-prey relationships by comparing the contributions of hake and other prey to the diets of sharks. The biomass contributions of hake to the diets of the three sharks was estimated using isotope mixing models combined with hake biomasses estimated from surveys. Given the common prey in their diets (Roel and Macpherson 1988; Yano 1991; Ebert 1994) and the overlapping depth distributions hake (Macpherson and Roel 1987; Bianchi *et al.* 1999; Burmeister 2001), I hypothesized overlapping diets and niche breaths for these hake and shark species. This study represents the first comparative investigation of the trophic relationships of sharks and hake in the northern Benguela Current ecosystem (Namibia) using stable isotope analysis.

6.2 Methods and materials

6.2.1 Field sampling

All shark, hake and prey samples were collected during hake surveys (January-February, 2011) as described in Chapter 2, section 2.1**.**

6.2.2 Laboratory analysis

All samples were measured for $\delta^{13}C$ and $\delta^{15}N$ at IsoEnvironmental CC Laboratory (Rhodes University, Grahamstown, South Africa) as described in Chapter 2, section 2.2.1. Relative trophic positions of species were calculated as described in Chapter 2, section 2.2.1.

6.2.3 Statistical analysis

Significant differences of $\delta^{15}N$ and $\delta^{13}C$ values among hake and shark species were determined using analysis of variance (ANOVA) followed by a Tukey HSD (Tukey Honest Significant Difference) *post hoc* test. Data were examined for normality and homogeneity of variance using Shapiro–Wilk and Levene tests. Violations of normality and homogeneity of the data were addressed using log transformations. The relationships between size (total length) and stable isotope values ($\delta^{13}C$, $\delta^{15}N$) were evaluated using correlation analysis. Calculation of isotope-based metrics such as $\delta^{15}N$ range (NR) indicated trophic diversity, $\delta^{13}C$ range (CR) represented the niche diversification at the base of a food web, and total area (TA) of the convex hull represented total amount of niche area occupied by species were done following Layman *et al.* 2007. The convex hulls shows how individuals are dispersed within isotope niche space (Jackson *et al.* 2012). Total area from a convex hull cannot, however, be compared between different sample sizes, as the convex hull area increases with sample size (Jackson *et al.* 2011). A standard ellipse area, corrected for small sample sizes (SEA_c), was calculated to account for variation in sample size (Jackson *et al.* 2011).

Feasible dietary contributions of hake and other potential shark prey were examined using a Bayesian isotope mixing model [SIAR (Stable Isotope Analysis in R)]. SIAR allows for the inclusion of isotopic signatures and fractionation together with their standard deviations into the mixing models (Parnell *et al.* 2010; Galva *et al.* 2012). The fractionation factors used in my models were 3.4‰ (±0.98) for $\delta^{15}N$ and 0.39‰ (±1.3) for $\delta^{13}C$ (Post 2002b). Although fractionation can vary among species (Vanderklift and Ponsard 2003; Caut *et al.* 2009), diet-tissue discrimination estimates for teleost fish have been successfully used to provide estimates for sharks (Logan and Lutcavage 2010).

The potential prey included in the models were identified based on previous stomach content studies of sharks (Yano 1991; Ebert *et al.* 1992). Each species of hake (*M. capensis* and *M. paradoxus*) was separated into two groups; medium-size hake (<40 cm) and larger hake (>40 cm). Hake that were less than 15 cm (juveniles) were not separated by species as they are trophically indistinguishable (Iitembu *et al.* 2012). Hake biomass estimates were derived from a 2011 hake survey (Kainge *et al.* 2011). All statistical analyses were done using R [R Core Team (2012), Vienna, Austria].

6.3 Results

6.3.1 Isotopic characteristics of hake and shark species

A total of 147 samples representing hake (*M. capensis* and *M. paradoxus)* and sharks *(Deania profundorum, Deania calcea* and *Deania squamosus*) was analysed to derive $\delta^{15}N$ and $\delta^{13}C$

isotopic signatures (Table 6.1). *Centrophorus squamosus* had the most enriched mean $\delta^{15}N$ (15.1 ‰) and most enriched mean $\delta^{13}C$ (-15.8‰). *Merluccius capensis* expressed the most depleted mean $\delta^{15}N$ (13.0 ‰), while *M. paradoxus* had the most depleted mean $\delta^{13}C$ (-16.6 ‰) (Figure 6.1).

Sizes of the species considered ranged from 22 to 65 cm for *M. paradoxus*, 16-69 cm for *M. capensis*, 50-88 cm for *D. profundorum*, 43-121 cm for *C. squamosus* and 49-110 cm for *D. calcea.* All the species had significant correlations between length (total length) and $\delta^{15}N$, with the exception of *C. squamosus* (R^2= 0.009, p-value = 0.71). *Merluccius paradoxus* (R^2= 0.0002, p-value = 0.92) and *C. squamosus* (R^2 = 0.15, p-value =0.12) showed no significant correlations between length and $\delta^{13}C$. *Centrophorus squamosus* had the highest average relative trophic level of 3.5, while *M. capensis* had lowest relative trophic level of 2.93 (Table 6.1, Figure 6.1). There was a significant difference among species' $\delta^{15}N$ (ANOVA, $p < 0.05$). A Tukey HSD *post hoc* test indicated that there was no significant difference between *D. profundorum* and *D. calcea (*p= 0.96*)*, between *M. paradoxus* and *D. calcea* (p= 0.12), and between *M. capensis* and *M. paradoxus (*p=0.13*)* (Figure 6.2). There was a significant difference ($p < 0.05$) among species with regard to their $\delta^{13}C$. Tukey HSD showed that the significant difference was between *M. paradoxus* and *C. squamosus* only *(*p=0.04) (Figure 6.3).

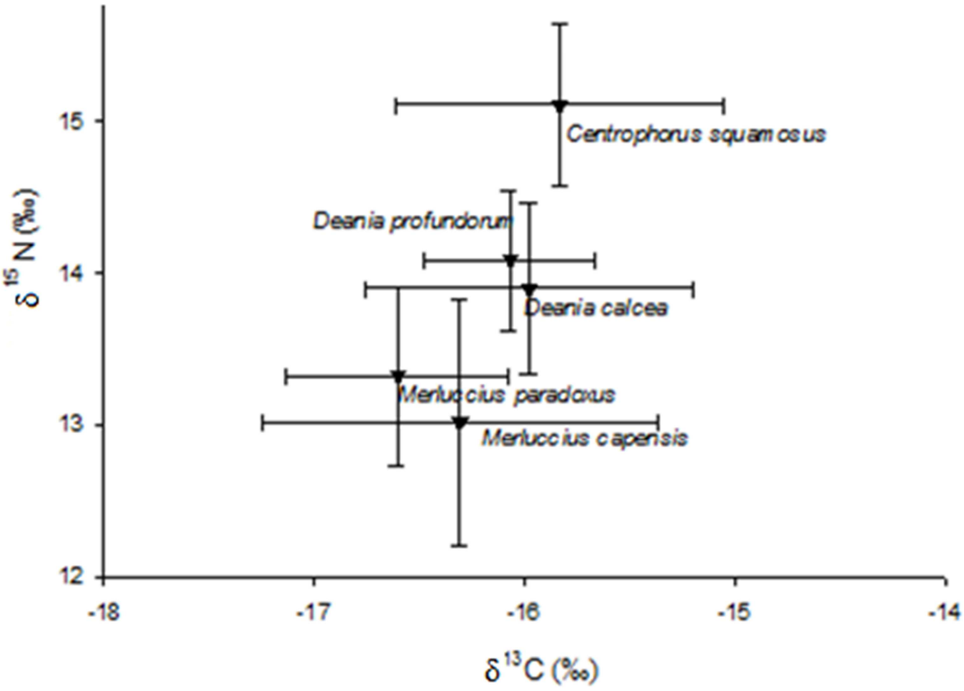

Figure 6.1: Bi-plot of means (triangles) and standard deviations (error bars) of δ^{15}N and δ^{13}C values for the three shark (*D. profundorum*, *D. calcea*, *C. squamosus*) and two hake species (*M. capensis* and *M. paradoxus*).

Table 6.1: Mean (±SD) values of stable nitrogen and carbon isotopes for sharks and hake, together with sample sizes (n), trophic level (TL) and C: N ratios.

Species	***n***	***TL***	**δ^{15}N**	**δ^{13}C**	**C:N**
M. paradoxus	*52*	3.1 (1.7)	13.32(0.59)	-16.60(0.53)	3.26(0.17)
M. capensis	*54*	2.93(0.24)	13.02(0.81)	-16.31(0.94)	3.21(0.07)
D. profundorum	*15*	3.24(0.13)	14.08(0.46)	-16.07(0.41)	2.61(0.07)
D. calcea	*9*	3.19(0.16)	13.90(0.57)	-15.98(0.78)	2.61(0.05)
C. squamosus	18	3.56(0.16)	15.11(0.53)	-15.83(0.78)	2.60(0.21)

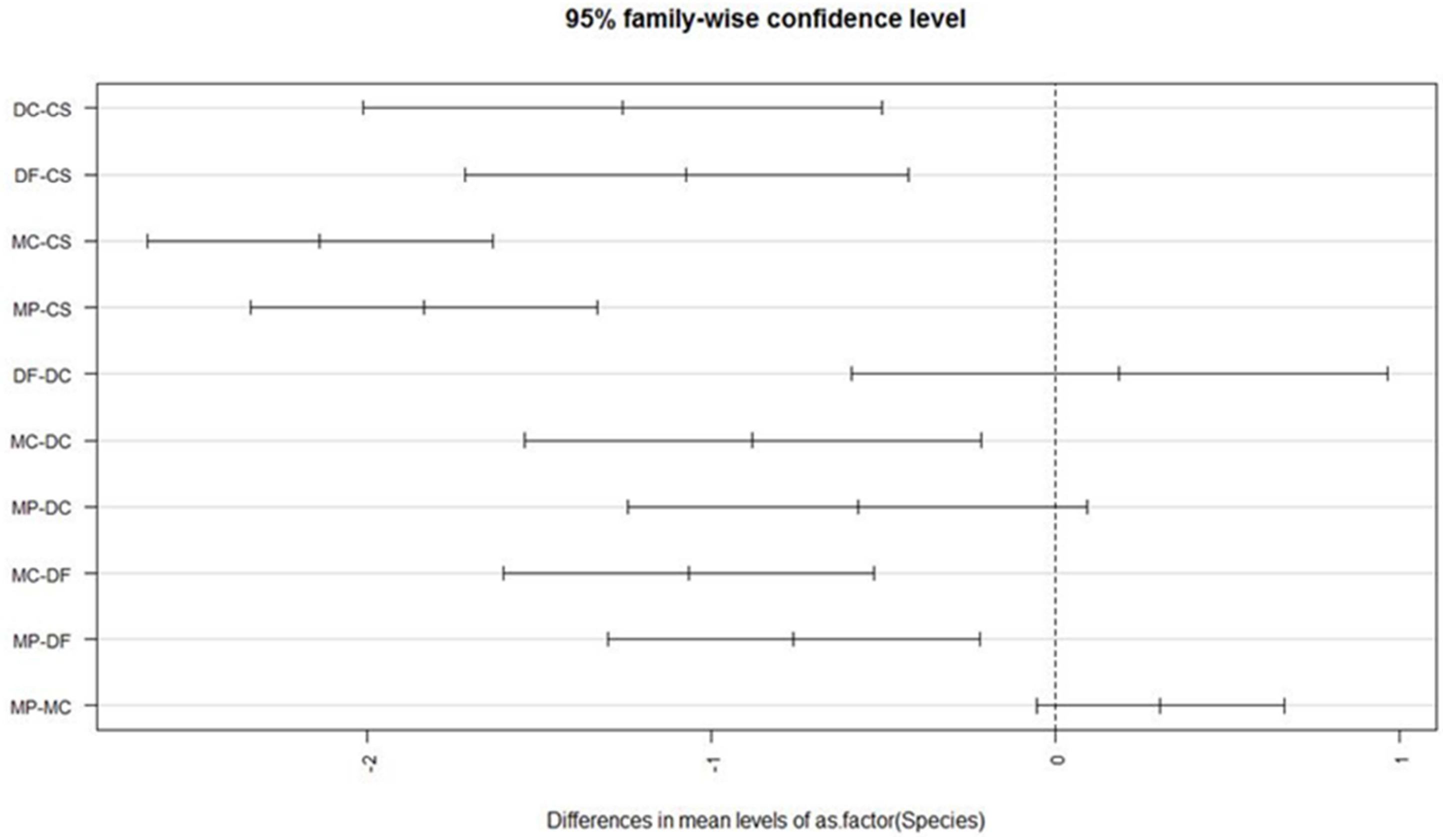

Figure 6.2: Tukey HSD test for δ^{15}N among species [*M. capensis* (MC), *M. paradoxus* (MP), *Deania profundorum* (DF), *Deania calcea* (DC) and *Centrophorus squamosus* (CS)]. Pairwise comparisons, with confident intervals of their means differences touch the zero line, indicates that their means differences is not significantly different from zero.

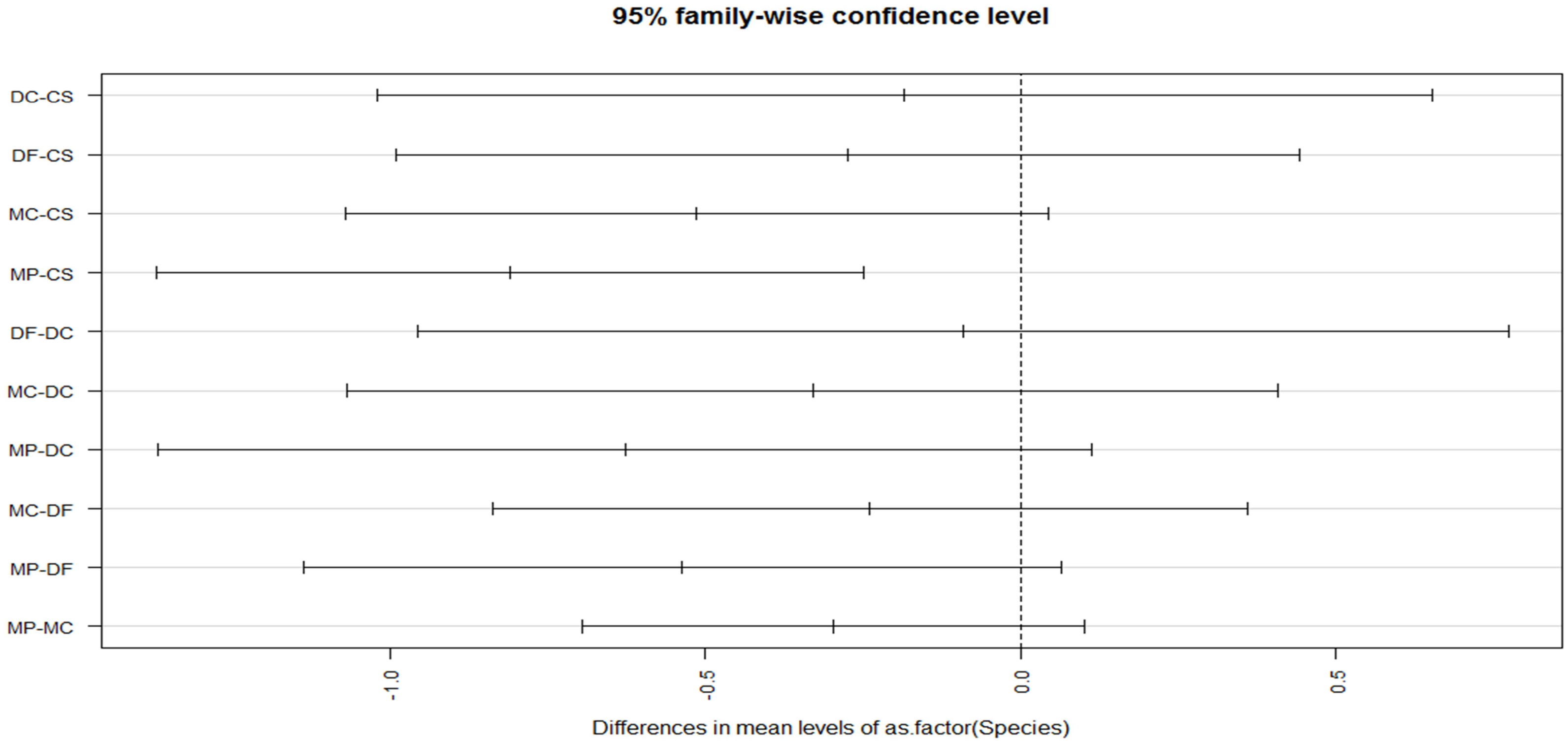

Figure 6.3: Tukey HSD test for δ^{13}C among species [*M. capensis* (MC), *M. paradoxus* (MP), *D. profundorum* (DF), *D. calcea* (DC) and *C. squamosus* (CS)]. Pairwise comparisons with confident intervals of their means differences touching the zero line, indicates that their means differences is not significantly different from zero.

6.3.2 Trophic niches and diets

Isotope-based population metrics indicated that *M. capensis* had widest δ^{15}N (4.2) and δ^{13}C ranges (4.20), while *D. profundorum* had the narrowest δ^{15}N (1.7) and δ^{13}C ranges (1.48). *Merluccius capensis* had the largest TA, with *D. profundorum* having the smallest (1.65) (Table 6.2). *Merluccius capensis* had the largest SEAc, while *D. profundorum* had the lowest (Table 6.2, Figure 6.4). The trophic niche of shark from *Deania* genus overlapped with that of both hake species (Figure 6.4)

Table 6.2: Carbon range (δ^{13} C R), nitrogen range (δ^{15} N R), the total area of the convex hull (TA) and standard ellipse area (SEAc) of hake and shark species. The number of individuals used to calculate the metrics are shown in parentheses.

Species	**δ^{15} N R**	**δ^{13}C R**	**TA**	**SEA$_c$**
M. capensis (54)	4.21	4.20	10.52	2.35
M. paradoxus (52)	3.02	3.97	6.26	0.97
Deania profundorum (15)	1.71	1.48	1.65	0.56
Deania calcea (9)	1.76	2.43	1.86	1.22
Centrophorus squamosus (18)	2.14	2.98	3.36	1.21

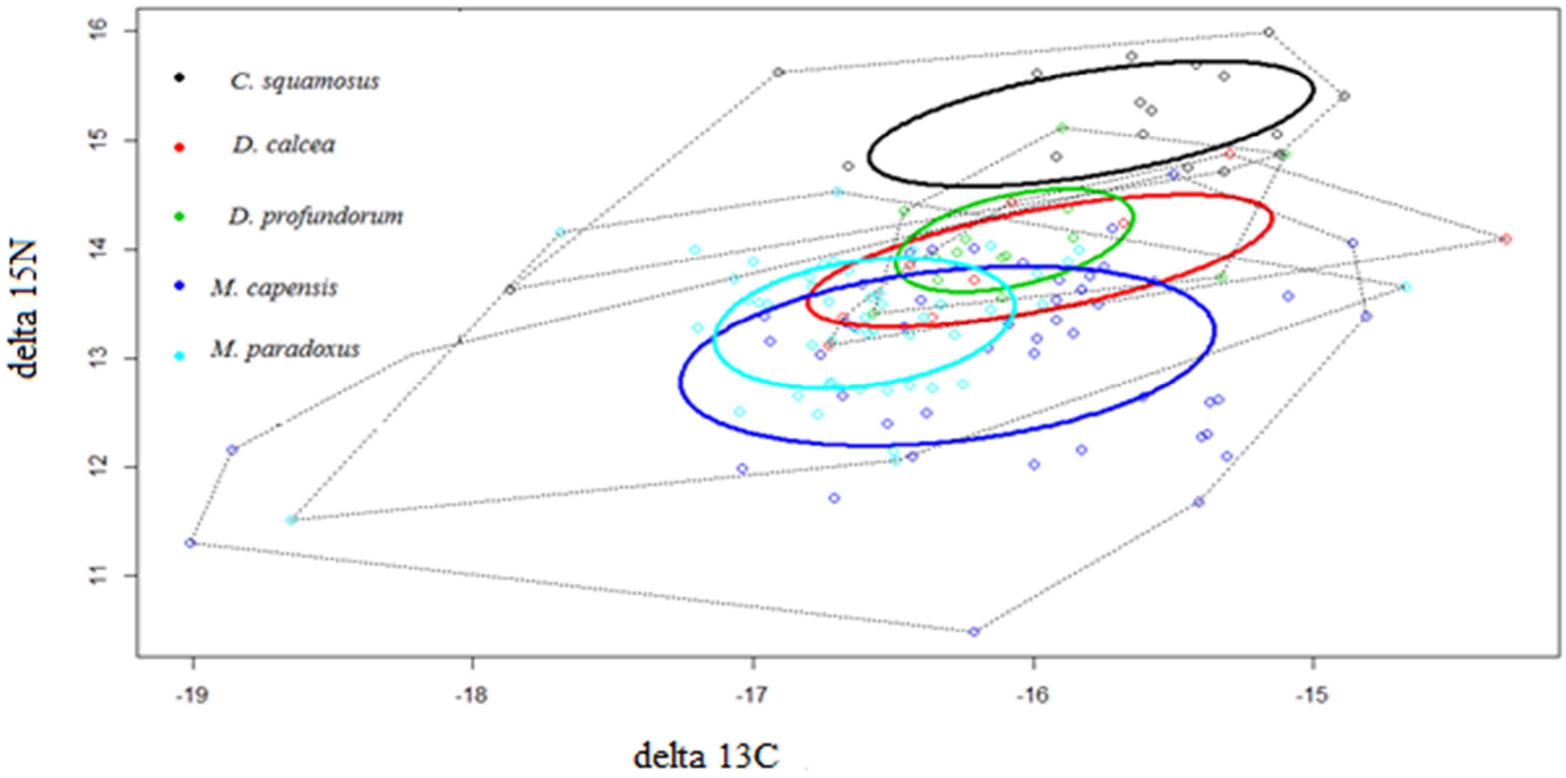

Figure 6.4: Trophic niche comparisons of *C. squamosus*, *D. calcea*, *D. profundorum*, *M. capensis* and *M. paradoxus* as depicted by convex hull (polygons) and SEAc (circle)**.**

A total of 201 samples representing 14 species or species group of fish, cephalopods and crustaceans (potential prey of sharks; Table 6.3) were analysed for isotopic signatures (δ^{15}N and δ^{13}C). Among the potential prey used in the model, *Caelorinchus simorhynchus* had the most enriched δ^{15}N (14.63‰), euphausiids (krill) had less enriched δ^{15}N (10.98‰) and most depleted δ^{13}C (-16.90‰). *Solenocera africana* had the most enriched δ^{13}C (-14.85‰). Potential prey trophic levels ranged from 3.4 for *Caelorinchus simorhynchus* to 2.33 for euphausiids (krill) (Table 6.3). Feasible contributions of different prey to the diets of the three sharks indicated that crustaceans were the major prey of *C. Squamosus* (around 40 %; Fig. 6.6). All prey contributed fairly equally to the diet of *D. calcea*, with slightly reduced contributions of larger *M. paradoxus, Nezumia micronychodon, Epigonus denticulatus* and *C. simorhynchus* (Fig. 6.7). The contributions of prey to the diet of *D. profundorum* were similar to that of *D. calcea* (Fig 6.8). Based on the 2011 hake biomass survey (Kainge *et al.* 2011) and SIAR dietary proportions (Fig 6.6-6.8), *C. squamosus* consumed 55 340.76 tonnes (4.9 %) of hake, D. *calcea* consumed 77 950.52 tonnes (7.0 %) and *D. profundorum* consumed 75 669.15 tonnes (6 %) (Table 6.4). The three sharks consumed 18% of the total hake biomass, of which 57 % was *M. capensis* (<40 cm; Table 6.4).

Table 6.3: Mean (±SD) values of stable nitrogen and carbon isotope ratios of muscle tissues from prey that were used in the SIAR model, with average C: N ratios, relative trophic level (TL) and sample size (*n*). $\delta^{13}C_{n,}$ represents lipid normalized values.

Species	***n***	**Taxon**	**Family**	***TL***	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{13}C_n$**	**C:N**
C. simorhynchus	7	teleost	Macrouridae	3.4	14.63(0.38)	-15.51(0.64)		3.12
E. denticulatus	4	teleost	Epigomidae	3.23	14.05(0.19)	-16.81(0.17)		3.23
Helicolenus dactylopterus	8	teleost	Scorpaenidae	2.76	12.45(2.38)	-16.09(1.72		3.18
M. capensis (>40 cm)	20	teleost	Merluccidae	3.0	13.26(0.76)	-15.76(0.52)		3.24
M. capensis (20-40) cm	34	teleost	Merluccidae	2.89	12.87(0.82)	-16.62(1.0)		3.19
M. paradoxus (> 40 cm)	26	teleost	Merluccidae	3.09	13.57(0.42)	-16.58(0.56)		3.31
M. paradoxus (20-40 cm)	26	teleost	Merluccidae	2.94	13.06(0.63)	-16.64(0.5)		3.21
myctophids	4	teleost	Myctophidae	2.81	12.62(0.51)	-18.99(0.46)	-15.60	6.78
N. micronychodon	12	teleost	Macrouridae	3.47	14.87(0.60)	-15.82(0.43)		3.12
Small hake (<15 cm)	11	teleost	Merluccidae	2.75	12.41(0.29)	-16.73(0.43)		3.23
A. varidens	19	crustacean	Aristeidae	2.58	11.80(1.05)	-16.18(0.99)		3.15
euphausiids(Krill)	16	crustacean	Euphausiacea	2.33	10.98(0.56)	-16.90(0.24)		3.29
S. africana	10	crustacean	Solenoceridae	2.56	11.71(1.09)	-14.85(0.70)		3.13
T. sagittatus	4	cephalopod	Ommastrephidae	2.86	12.51(.088)	-16.83(0.24)		3.26

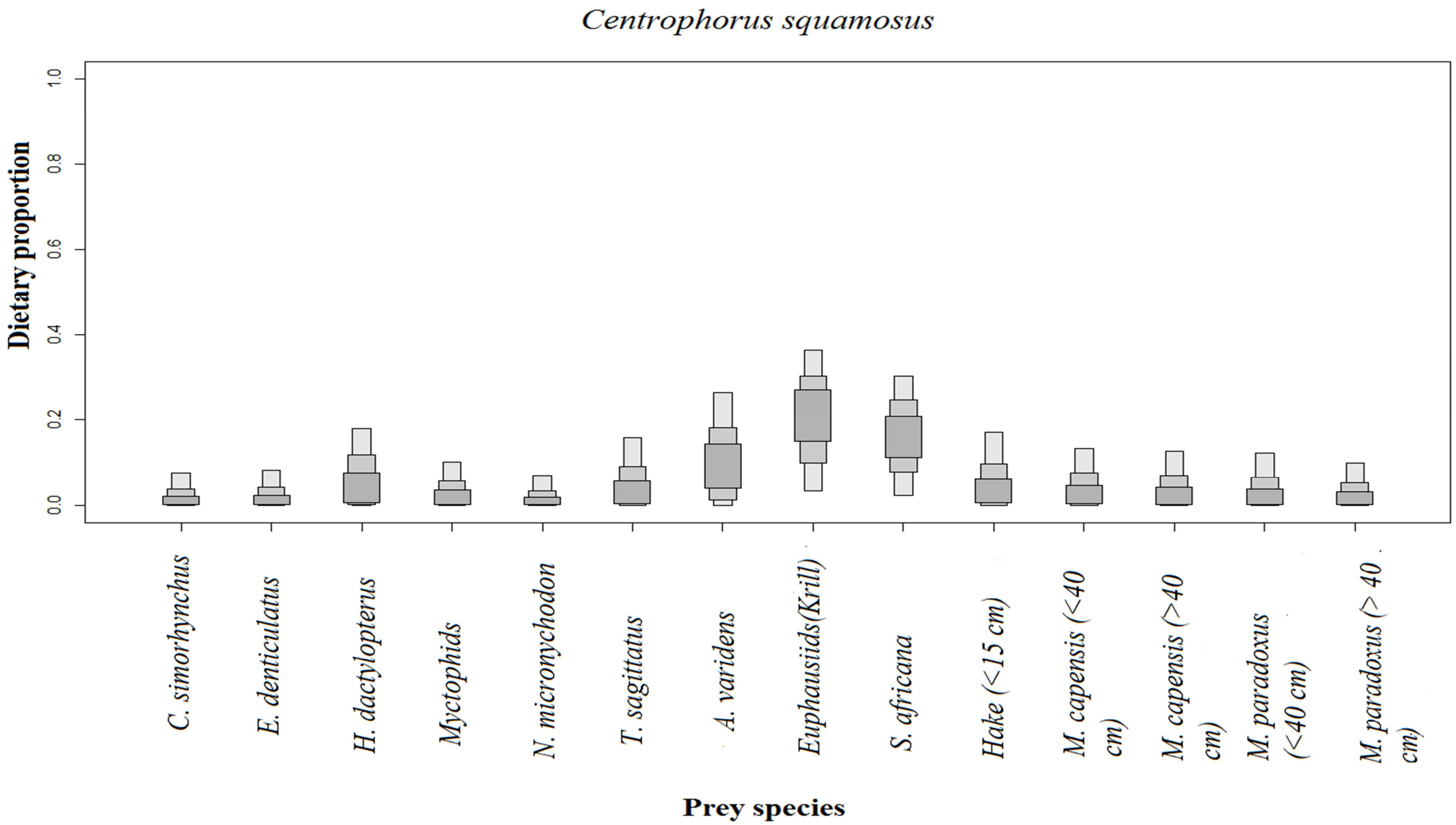

Figure 6.5: Contributions of the potential prey to the diet of *C. squamosus*, as determined by a SIAR mixing model. Each box and whisker shows 95%, 75% and 50% credibility intervals.

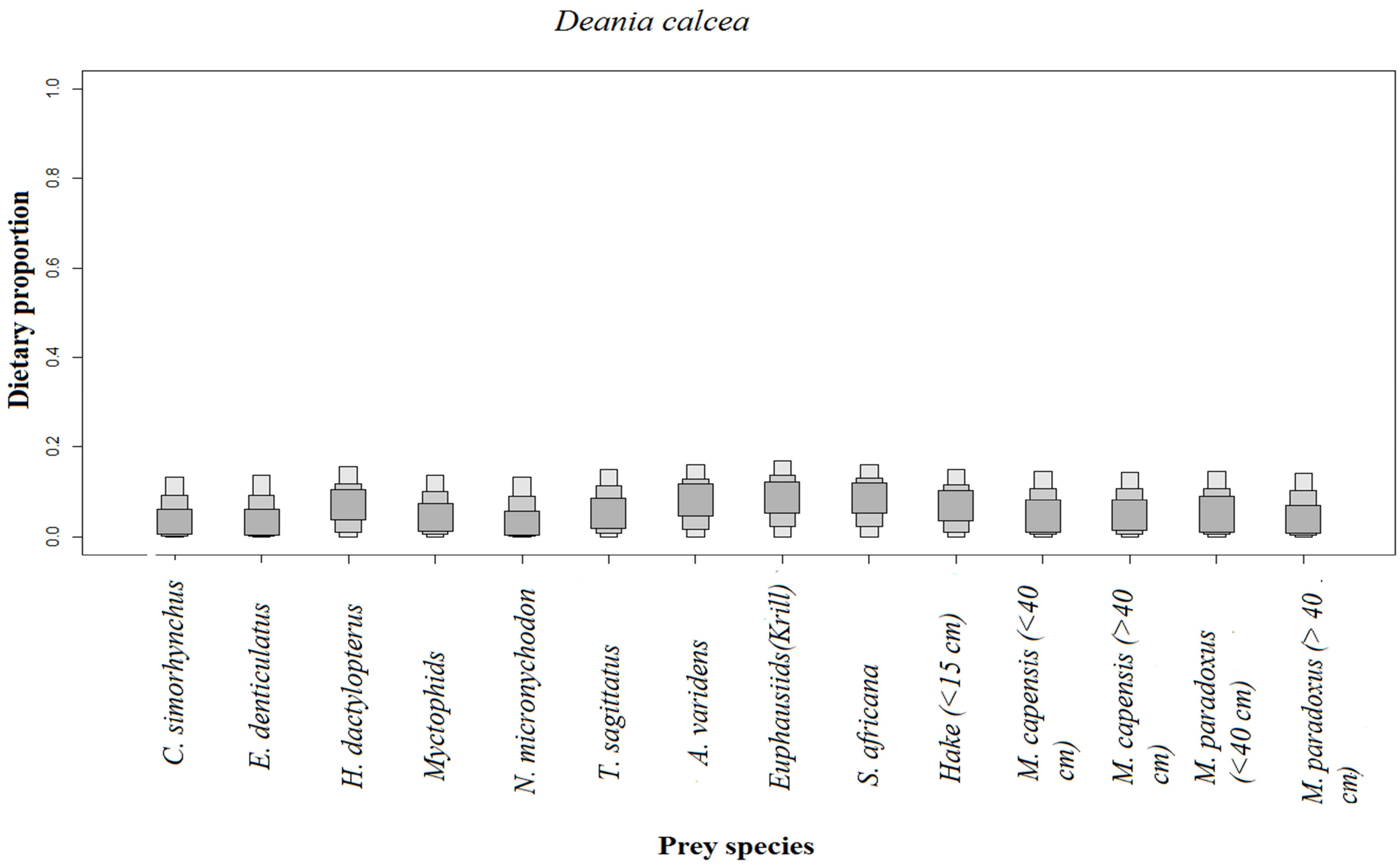

Figure 6.6: Contributions of the potential prey to *D. calcea* diet as determined by SIAR mixing model. Each box and whisker shows 95%, 75% and 50% credibility intervals.

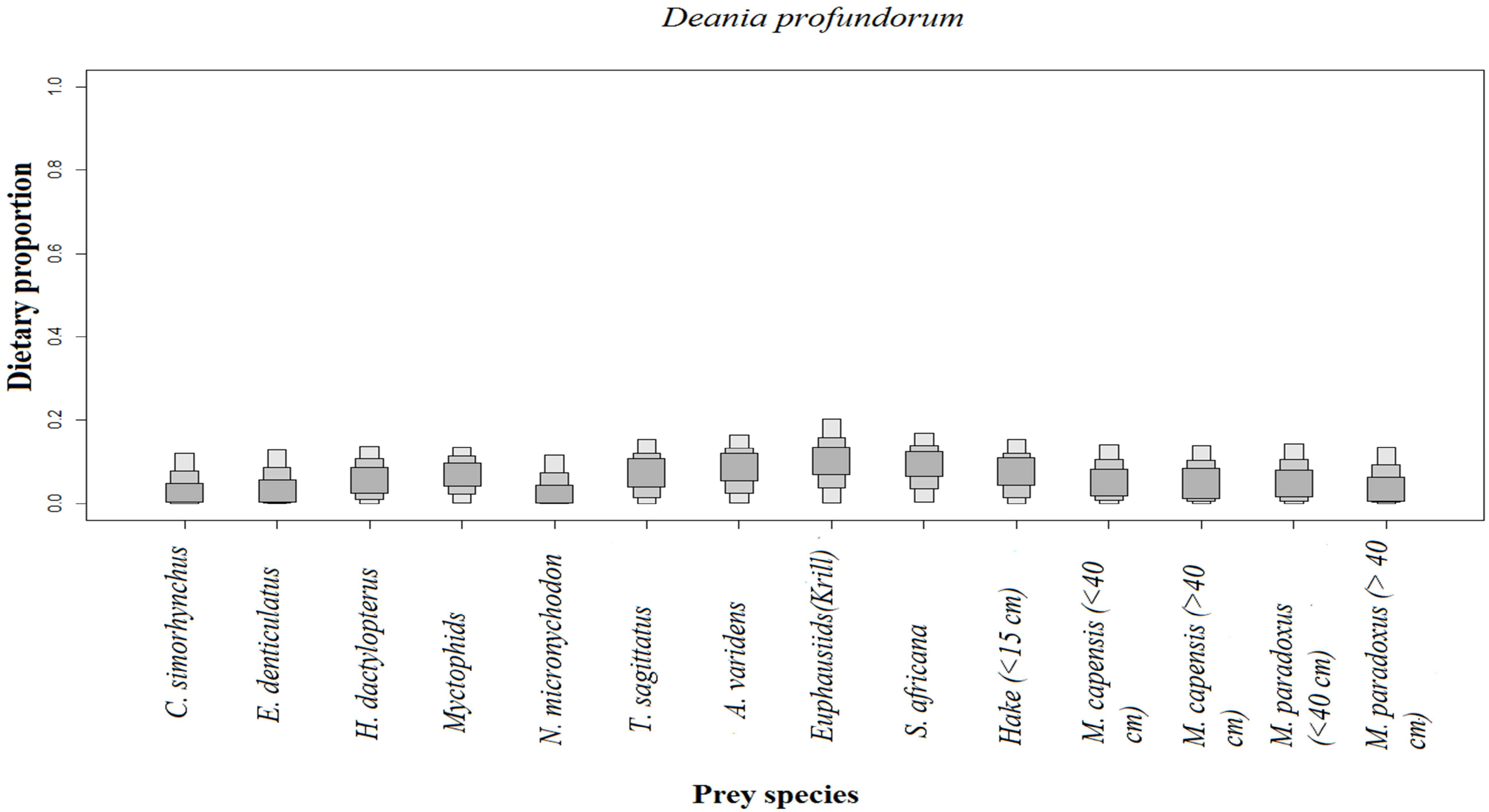

Figure 6.7: Contributions of the potential prey to *D. profundorum* diet as determined by SIAR mixing model. Each box and whiskers shows 95%, 75% and 50% credibility intervals

Table 6.4: Estimated mean contributions of hake biomass (tonnes per year) to the diets of sharks (*C. squamosus*, *D. profundorum* and *D. calcea*) based on a 2011 biomass estimate and dietary proportions from SIAR models.

	Hake biomass	*C. squamosus*	*D. calcea*	*D. profundorum*	**Total biomass**
M. capensis (<40 cm)	628 003.5	32 904.65	44672.61	43125.99	**120703.25**
M. capensis (>40 cm)	235 139.8	11 198.42	16306.98	15721.94	**43227.34**
M. paradoxus (<40 cm)	160 592.4	7 502.44	11081.39	11093.78	**29677.61**
M. paradoxus (>40 cm)	69 571.7	2 556.95	4567.03	4322.04	**11446.02**
Small hake *(< 15 cm*	17 889.4	1 178.31	1322.50	1405.41	**3906.22**
Total biomass	**1 111 196.8**	**55 340.76**	**77 950.52**	**75 669.15**	**208 960.48**

6.4 Discussion

The ranges of $\delta^{15}N$ and $\delta^{13}C$ values of sharks (*C. squamosus, D. calcea,* and *D. profundorum)* and hake (*M. capensis* and *M. paradoxus*) off Namibia support the hypothesis that these different species have overlapping trophic niches and feeding ranges. Sharks have more enriched $\delta^{15}N$ signatures than hake, but similar $\delta^{13}C$ signatures, showing that different shark prey probably have similar $\delta^{13}C$ signatures. Of the three sharks, those in the *Deania* genus consume the greatest hake biomass. Sharks also consumed more *M. capensis* than *M. paradoxus*, likely a result of their differing population abundances in Namibian waters.

Centrophorus squamosus fed at the highest relative trophic level compared to the other species analysed (mean $\delta^{15}N$ of 15.1 ‰, trophic level 3.5), with nitrogen isotope values being significantly different from all other shark and hake species in this study. The two sharks from the *Deania* genus *(D. profundorum* and *D. calcea)* were not significantly different from each other in terms of $\delta^{15}N$, an indication that they fed on prey from similar trophic levels. *Merluccius capensis* and *M. paradoxus* were not significantly different from each other in terms of $\delta^{15}N$, showing that there is dietary overlap between the two species. This similarity confirms stomach content findings from previous studies (Roel and Macpherson 1988); however, as indicated in Chapter 3, the two hake experience different shifts in their diets through their development. *Merluccius capensis,* with the most depleted mean $\delta^{15}N$ (13.0 ‰) (TL=2.93), fed on prey at lower trophic levels compared to all other species. *Merluccius capensis* lives at shallower depths (Burmeister 2001) than *M. paradoxus*, and it possibly fed on prey that inhabit the continental shelf. Inhabitants of the continental slope have more depleted $\delta^{15}N$ values (Yoshii *et al.* 1999). The prey base of *M. paradoxus* overlapped with that of *D. calcea,* as no significant differences in their $\delta^{15}N$ was detected. The above is possibly because large *M. paradoxus* show increased piscivory in their diets relative to *M. capensis* (Iitembu *et al.* 2012).

In terms of $\delta^{13}C$, *C. squamosus* had the most enriched mean values (-15.8‰), an indication of dependence on benthic prey. However, the only significant difference in $\delta^{13}C$ was between *M. paradoxus* and *C. squamosus.* These results showed that *M. paradoxus* and *C. squamosus* fed on prey which differed in carbon sources. *M. paradoxus*, which had the most depleted mean $\delta^{13}C$ (-16.6 ‰), possibly having fed on more pelagic prey, which have more depleted $\delta^{13}C$ values relative to benthic-feeding fish (France 1995a; Davenport and Bax 2002; Leloch *et al.* 2008).

The relatively enriched δ^{13}C in benthic species is because of their dependence on organic carbon modified through degradation and microbial recycling processes (Fry and Wainright 1991; Hobson *et al.* 1995). Although measuring the trophic interaction strength between species is highly problematic (Wootton and Emmerson 2005), however given the diet and isotopic information provided here, the trophic interaction strength between *M. paradoxus* and *C. squamosus* is likely relatively low.

All sharks and hake, with the exception of *C. squamosus*, had significant positive correlations between total length and δ^{15}N, an indication of ontogenetic shifts in their feeding habits (Araújo *et al.* 2011). Positive correlations between total length and δ^{15}N are common in many marine organisms (Deudero *et al.* 2004) including hake (Iitembu *et al.* 2012) and sharks (Estrada *et al.* 2006). Correlation analysis of δ^{13}C and length failed to show relationships in *M. paradoxus* and *C. squamosus*, indicating some degree of specialization of the two species. While fish can feed on a wide range of available prey, the community biomass may be largely reliant only on a relatively few abundant prey taxa (Al-Habsi *et al.* 2008). However, conclusions on specialisation cannot be based on isotope data; further analyses like stomach content analyses of sharks need to be conducted to justify these conclusions.

Merluccius capensis, which had wider δ^{15}N and δ^{13}C ranges than the other species, possibly fed on prey from various trophic levels and multiple basal resources. All of the sharks expressed narrower δ^{15}N and δ^{13}C ranges than the hake, an indication of narrow feeding ranges in the sharks. The total isotopic area, which represents total amount of niche area occupied by a species (Layman *et al.* 2007), was also narrower for the sharks compared to the hake. Some species of sharks exhibit some form of specialized feeding (Taylor and Bennett 2008; Matich *et al.* 2011). However, no sharks in my study occupied unique isotopic niche areas, although mean isotopic values differed between species.

A number of sharks prey were included in the SIAR model and were chosen based on published gut content accounts (Yano 1991; Ebert *et al.* 1992; Ebert 1994). The inclusion of many prey avoids SIAR's missing source biases (Parnell et al. 2013) and reduces the effect of the compositional data's constant sum constraint (Aitchison 1986; Kucera and Malmgren 1998). Among the potential prey, C. *simorhynchus* had the most enriched mean δ^{15}N (14.63 ‰) (TL= 3.4) and euphausiids the most depleted δ^{15}N (10.98) (TL = 2.3), forming the endpoints of the trophic spectrum. In terms of $\delta^{13}C$, euphausiids had the most depleted $\delta^{13}C$ (-16.90 ‰), an indication that it inhabited more pelagic waters while *S. africana*, with the enriched $\delta^{13}C$ (-14.85 ‰), exploits more benthic systems.

Crustaceans were the major prey of *C. squamosus* (around 40 %), with low contributions from both hake species (around 4 %). Crustaceans tend to be more important in the diets of squalid sharks than in other shark species (Vaudo and Miami 2011). These results differed from previous stomach content findings by Ebert *et al.* (1992a), which indicated that cephalopods were the most dominant prey group for *C. squamosus*. The only cephalopod in this study (*T. sagittatus*) was the third most feasible contributor after crustaceans (euphausiids, *A. varidens* and *S. africana)* and a teleost *(H. dactylopterus).* However, findings in Ebert et al. (1992a) were limited by the number of stomachs analysed (18), making it difficult to compare their findings to my isotope results.

The two *Deania* shark species (*D. calcea* and *D. profundorum*) had similar prey contributions to their diets, with all of the species contributing less than 7 % each. It is conceivable that Deania sharks feed on a variety of prey and are less specialized than *C. squamosus*. Hake (*M. paradoxus* and *M. capensis*) contributed more to the diet of the two *Deania* sharks than *C. squamosus.* The dietary contributions of hake to shark diets were not, however, different from the contributions of other prey. There was also a reduction in feasible dietary contributions of larger *M. paradoxus, N. micronychodon, E, denticulatus* and *C. simorhynchus* to shark diets in comparison to other species considered. Based on the 2011 hake survey biomass estimates (Kainge *et al.* 2011), the three sharks in this study consumed an estimated 18% of the hake total biomass (208 960.48 tonnes), of which 57 % was *M. capensis* (<40 cm). The greater consumption of *M. capensis* was mainly because it is more abundant in Namibian waters than *M. paradoxus* (Kainge *et al.* 2011).

The similarities between the diets of *D. profundorum* and *D. calcea* are probably related to the morphological similarities in the two body forms and their feeding structures (Bianchi *et al.* 1999; Ferry-Graham *et al.* 2002). My results therefore support the hypothesis of a specialized diet for *C. squamosus* and more generalized diets in *D. calcea* and *D. profundorum*. Some sharks specialize on the consumption of teleosts, while other specialize on crustaceans (Braccini 2008). Sharks from the genus *Deania* are considered as principally fish eaters in the north eastern Atlantic Ocean (Mauchline and Gordon 1983). It has been noted based on FA profiling and gut content analyses of dogfish that those occupying a broad depth and bathymetric distribution were recognized as more generalist feeders, whilst other species occupying upper-slope had diet dietary selectivity for crustaceans (Pethybridge et al. 2011).

The three shark species occupied a relatively small area of isotopic niche space relative to the two hake species. A narrower isotopic niche in sharks can indicate feeding specialization that can reduce competition with hake; however, the individual variability in isotopic values shows high degrees of overlap among these predators. This study carries limitations inherent in the use of stable isotopes in ecological studies, such as the potential errors involved in using average trophic fractionation factors of 3.4‰ for $\delta^{15}N$ and 0.39‰ for $\delta^{13}C$ (Post 2002b). Experimental work has demonstrated that fractionation can vary among consumers (Vanderklift and Ponsard 2003; Caut *et al.* 2009). Future studies should therefore focus on measuring species specific trophic fractionation factors. While data in my study allowed for some inferences on the trophic relationships of several predators, I recommend that a larger study with increased sample sizes for predators and prey across time and space. The increase in sample size can increase the precision of population level isotopic niche calculations (Syväranta et al. 2013) and possibly the output of isotopic mixing model, as it will cover the distribution and range of the population isotope values.

CHAPTER 7: TROPHIC RELATIONSHIPS OF HAKE SPECIES (*MERLUCCIUS CAPENSIS* AND *M. PARADOXUS*) FROM THE NORTHERN BENGUELA CURRENT ECOSYSTEM (NAMIBIA): A SYNTHESIS

7.1 Introduction

In this study, I aimed at increasing our understanding of the trophic relationships of two hake species (*M. capensis* and *M. paradoxus*) using time integrating trophic biomarkers. Earlier inferences on the trophic relationships of hake have been based mainly on stomach content analyses (Macpherson 1983; Roel and Macpherson 1988; Punt *et al.* 1992; Traut 1996), a method with several inherent limitations. The trophic relationships of hake relative to each other, their known prey and top predators (demersal sharks) in the northern Benguela Current ecosystem (Namibia) were investigated using stable isotopes and FA signatures of their muscle tissues. The combined use of stable isotope and FA signatures can shed new light on trophic relationships of marine species, particularly in situations where stomach content analyses alone were insufficient (Alfaro *et al.* 2006; El-Sabaawi *et al.* 2009; Stowasser *et al.* 2009). My study provided new information on hake species' ontogenic trophic shifts (Chapter 3), the contributions of different prey to hake diets (Chapter 4), hake species dietary differences (Chapter 5), and some aspects of their trophic relationships to demersal sharks (Chapter 6). It also demonstrated the added value of using trophic biomarkers to study trophic ecology of marine species. The results will aid the consideration of trophic interactions in fisheries management approaches, one of the requirements for the successful implementation of the ecosystem approach to fisheries management in Namibian waters (Shannon *et al.* 2006; Cochrane *et al.* 2009).

In the following sections, the trophic relationships of the hake species (*M. capensis* and *M. paradoxus*) in the Northern Benguela current ecosystem (Namibia) are discussed in light of new knowledge from trophic biomarkers. This is followed by a brief discussion on the importance of incorporating information of trophic relationships in fisheries management approaches. The importance of new knowledge about species trophic relationships to the successfully implementation of an ecosystem approach to fisheries (EAF) management (Garcia and Cochrane

2005; Shannon *et al.* 2010) is also briefly discussed. Finally, recommendations are made for future studies to complement the findings of this study.

7.2 Trophic relationships of hake (*M. capensis* and *M. paradoxus*) off Namibia

Both hake species become piscivorous with increasing size (Macpherson 1983; Roel and Macpherson 1988; Punt *et al.* 1992; Traut 1996), but their ontogenic trophic relationships were not known. This study showed that both *M. capensis* and *M. paradoxus* had a significant positive relationships size and nitrogen isotopes (δ^{15}N; Chapter 3), an indication of their ontogenic trophic shift. However, the slopes of the two species' ontogenic trophic shifts were significantly different, with *M. paradoxus* displaying a steeper trophic shift. The differences suggest that, although the two species are sympatric, there is resource partitioning with growth, which can reduce food competition between them. A significant increase in carbon isotope (δ^{13}C) with size, which was observed in *M. capensis* but not in *M. paradoxus*, also suggest the former changes to prey with different carbon source as well. This study showed that there is greater trophic interaction of smaller hake of both species (20–39 cm), which were trophically indistinguishable (Chapter 3). The larger hake were trophically different from each other, suggesting that larger *M. paradoxus* feed at higher trophic levels than larger *M. capensis* (Chapter 3 and 4). Food web models from the Benguela region estimated a higher trophic level for hake species [4.0 for small and 4.5 for large *M. capensis*, and 4.1 for large M. *paradoxus* (Roux and Shannon, 2004; Watermeyer et al., 2008; Shannon and Jarre, 1999)]. The ecosystem model results put them at the trophic levels which are similar to large pelagic fish, seals and seabirds (Wilhelm *et al., in* review). Interestingly, this study suggested the possibility of feeding competition between *M. paradoxus* and sharks from the genus *Deania*, as they had overlapping feeding ranges (Chapter 6). The above suggest that the population dynamics of the hake are possibly affected by different prey dynamics, whose management will require a different approach.

Feasible contributions of different prey to the diets of the two hake, as determined by Bayesian isotope-mixing models, indicated that the two species have overlapping diets (Chapter 4). The mixing model findings are consistent with results from stomach content analyses (Payne *et al.* 1987; Roel and Macpherson 1988) and the findings of their FA signatures (Chapter 5). My findings suggested that *S. microlepsis, P. martia, C. agazizi*, myctophids and euphausiids are important prey of the two hake species (Chapter 4). The highest contributor to the diet of *M. paradoxus* varied with the length of the predator, while the diet of *M. capensis* was dominated by myctophids at all length classes (Chapter 4). Thus myctophids appeared more important to the diet of *M. capensis* than to the diet of *M. paradoxus.* Fatty acid signatures also suggested that the flying squid (*T. sagittatus*) had greater influences on the diet of *M. paradoxus* than on the diet of *M. capensis* (Chapter 5). The influence of different key prey to the diets of the two species is also reflected in the neutral FA signatures, which were significantly different from each other (Chapter 5). This study provides strong evidence that, although the two species have overlapping feeding ranges, they have different prey that are key to their respective diets. The importance of the prey to their diets is possibly also influenced by temporal variations in quantity and quality of prey dietary resources, as reflected by significant differences between samples from the two collection periods (Chapter 5).

High rates of cannibalism have been reported in the two hake species, with studies based on stomach content analyses indicating that it may account for >70% (prey mass) of hake diet (Payne *et al.* 1987; Roel and Macpherson 1988; Traut 1996). The isotope-mixing model results indicated that it accounted for less than 6% and 4% in the diets of *M. capensis* and *M. paradoxus* respectively (Chapter 4). Isotope-based estimates of prey dietary contributions suggest that previous gut content analyses overestimated the time integrated cannibalism rates. The differences can, however, be consequences of the different methods used, with stomach content analyses representing snapshots of recent feeding (Cortés 1997), while isotopes represented time-integrated views of the hake diets. The higher levels of cannibalism observed from gut content results may also be due to prey patch characteristics, such as local densities of hake at the times of feeding (Benoit-Bird *et al.* 2013). My results therefore suggest that in the long term, cannibalism might not be the main cause of natural mortality of hake, as previously thought (eg. Lleonart *et al.* 1985). While efforts have been made to include all potential prey of hake, mixing models results suffer from missing source biases as result of the compositional nature of their results (Parnell *et al.* 2013). The mixing models results are also limited by the constant sum constraint (Aitchison 1986; Kucera and Malmgren 1998), with poor estimation of one prey dietary proportion leading to the others being poorly estimated too (Parnell *et al.* 2013).

Other than the stomach content analyses on squalid sharks, which indicated that they feed on hake (Ebert *et al.* 1992), shark trophic relationships relative to hake are not well understood. Stable isotope-based estimates of shark diets indicated that hake are not the main prey of *C. squamosus,* whose diet was dominated by crustaceans. However, there was an increased contribution of hake to the diets of *Deania* sharks (Chapter 6). The fact that the three sharks can potentially consume upwards of 18 % (208 960 tonnes) of the total hake biomass (1 111 196.8 tonnes, 2011 biomass estimate) suggests that sharks are one of the main contributors to the natural mortality of hake. Equally, it is also plausible that any change in hake biomass will have an impact on the population of sharks in this genus. Population estimates of sharks indicate that they have been declining globally (Heithaus *et al.* 2008), and it is possible that the declining biomass of hake observed in Namibian waters (Rashid and Vasconcellos 2000) had an impact on the shark populations because of their predator -prey relationships (Chapter 6). In the light of this possibility, it will be interesting to compare the population trends of sharks and hake over time. Although sharks are top predators, my study indicated that hake and shark species fed on prey from similar basal resources. The similarities of basal sources are possibly a result of intensive and persistent upwelling in Namibian waters, resulting in homogeneity of basal resources across the areas studied. The influence of the upwelling regime is between 150 and 200 km wide (Duncombe Rae 2005), with the filamentous mixing area that can extend to 625 km offshore (Campillo-Campbell and Gordoa 2004). The two hake species, however, had wider ranges of carbon sources than did the three shark species, and greater trophic diversity in their feeding patterns. This study suggested that, in comparison to the hake, sharks are more specialized in their feeding.

7.3 Trophic relationships and fisheries management

There is a common understanding among fishery managers and scientists that new alternative approaches are needed to manage fisheries resources at ecologically sustainable harvest levels. Recognizing the need for management approaches based on integrated ecosystem considerations, fisheries management authorities are now moving towards an ecosystem approach to fisheries (EAF) management (Garcia *et al.* 2003; Roux and Shannon 2004; Garcia and Cochrane 2005; Cochrane *et al.* 2009; Paterson and Petersen 2010; Paterson *et al.* 2010; Volckaert 2013).

However, adopting EAF requires, among other things, an understanding of the trophic ecology of fish communities in the food web (Byron and Link 2010). Understanding trophic relationships in an ecosystem will aid EAF implementation by closing gaps in our knowledge of vital ecological processes and linkages (Shannon *et al.* 2010). While trophodynamic interactions have been recognized as important in the implementation of EAF, these interactions are poorly known in most cases (Cury *et al.* 2005b). Attempts to include ecosystem considerations in fisheries management is not new to fisheries science (Lotka 1925; Holling 1965; Berryman 1992). However knowledge of trophic relationships of species is still not explicitly considered in fisheries management (Link 2002b; Simons *et al.* 2013), which is limitation of currently used fisheries management approaches (Pauly *et al.* 2000, 2002, 2003; Cury *et al.* 2005a; Sibert *et al.* 2006).

The development of ecosystem models have further improved our ability to study trophic interactions in marine ecosystems (Jarre-teichmann 1998; Pauly *et al.* 2000; Gascuel and Pauly 2009), but to be meaningful, ecosystem models need to be parameterized based on sound empirical data (Jarre-teichmann 1998; Roux and Shannon 2004). Results in this study (Chapters 3, 4, 5, and 6) provide trophic information which is necessary for understanding and quantifying the trophic interactions in Namibian waters. These findings will allow for refinement of parameters used in ecosystem models (Pauly *et al.* 1998) and stock assessment models (A'mar *et al.* 2010), thus promoting a science-based implementation of EAF management of hake and other fisheries resources in Namibian waters. While it cannot be assumed that inclusion of trophic interactions in management procedures will lead to improved fisheries regulation (Punt and Hilborn 1994), knowledge of trophic relationships among marine species remains crucial to the management of our marine resources with greater confidence (Yodzis 1994; Mueter and Megrey 2006).

7.4 Future research

The results of my study have demonstrated the usefulness of trophic biomarkers to investigate trophic relationships of hake in the northern Benguela ecosystem (Namibia). Trophic interactions are however highly complex, non-linear and can change with time (Jarre-teichmann *et al.* 1998; Duarte and Garc 2004; Croll *et al.* 2005; Roux *et al.* 2013). Thus there is great need for long-term studies of these predators and their prey which incorporate both small and large-scale temporal and spatial sampling within the Northern Benguela ecosystem.

The use of stable isotopes in this study carried the uncertainties associated with fractionation factors and isotopic turnover rates, which can vary among species and size. Future research should focus on determining species-specific fractionation factors and isotopic turnover rates. Accurate use of fractionation factors and turnover rates is crucial for the findings of isotope based trophic studies to help direct future research and improve parameters (e.g. natural mortality) for stock assessment models.

The use of FAs to investigate trophic relationships at higher trophic levels is also limited by the lack of understanding of how individual FAs change through elongation and desaturation processes occurring throughout a food web. To use FAs with confidence in dietary studies, it is important to understand the direct effects of diet on FA compositions and turnover in the tissues of predators. Future research should include laboratory studies to understand the turnover and deposition of FAs in hake tissues. The utilization of FAs as dietary tracers in laboratory studies will then be used to understand processes occurring in trophic food webs, so that we may trace paths of FAs to hake in open-ocean systems.

While the use of trophic biomarkers provided a time-integrated view of hake diet, it missed recent feeding activities that are accessible using stomach content analyses. A study involving comprehensive stomach content analyses, in combination with trophic biomarkers, is recommended to accurately assess dietary habits of hake species. Such a study could also be conducted in conjunction with a prey biomass survey, to answer questions related to prey patch characteristics; for example, prey local densities at times of predator feeding. The prey biomass survey results could then be used with isotope mixing models and stomach content analyses to make inferences on the importance of particular prey to the diet of hake.

Major changes have occurred in the waters off Namibia (Benguela region) that included spatial changes in the distribution of small pelagic fish (Shannon *et al.* 2003; Shannon and Cury 2004), and increases in biomass of other species such as jellyfish (Flynn *et al.* 2012; Roux *et al.* 2013) and gobies(Utne-palm *et al.* 2010). Whether these changes are as result of regime shift or climate changes it is uncertain. The collection of biochemical data (such as the one herein) need to be continued so that we can create long term data set. It has been suggested that biochemical data show promise in determining species response to climate change or regime shift and they can potentially move us beyond the reliance on conventional techniques (Parrish et al. 2013). Additionally biochemical tracers have a role to play in future applications of ecosystem management, especially in assessment and monitoring. These ecological data are integrated and extracted from the tissues of marine organisms in predictable ways. However, they are complex and difficult to explain to managers who are more familiar with traditional emperical data. Attempt should be made to simplify their interpretations so that practical and realistic management measures can be based on these data.

My study represents the first that has combined usage of stable isotope and fatty acid analyses to look at trophic relationships of *M. capensis* and *M. paradoxus* in the northern Benguela Current (Namibia). An extension of these analyses throughout hake distribution range would provide considerable insights into the trophic dynamics of hake and other oceanic predators.

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APPENDIX

Appendix 1: Fatty acid (FA) compositions of *Merluccius capensis* and *Merluccius paradoxus*

	January 2011		December 2011	
	M. capensis(n=25)	*M. paradoxus (n=15)*	*M. capensis(n=34)*	*M. paradoxus (n=36)*
Saturated Fatty Acids (SFAs)				
14:0	1.11 ± 0.93	1.12 ± 0.97	2.23±0.90	1.45±0.74
i-15:0	0.00 ± 0.00	0.00 ± 0.00	0.10±0.07	0.06±0.07
15:0	0.14 ± 0.15	0.10 ± 0.12	0.39±0.15	0.34±0.16
16:0	19.44 ± 3.72	18.69 ± 2.85	20.21±1.62	20.44±3.47
i-17:0	0.00 ± 0.00	0.02 ± 0.07	0.42±0.19	0.26±0.16
ai-17:0	0.13 ± 0.16	0.14 ± 0.17	0.13±0.13	0.07±0.10
17:0	0.35 ± 0.25	0.15 ± 0.19	0.60±0.22	0.46±0.19
18:0	5.51 ± 1.39	4.54 ± 1.26	6.26±2.07	5.24±1.68
20:0	0.00 ± 0.00	0.00 ± 0.00	0.30±0.17	0.23±0.69
21:0	0.00 ± 0.00	0.00 ± 0.00	0.24±.24	0.15±0.17
22:0	0.00 ± 0.00	0.00 ± 0.00	0.03±0.07	0.23±1.24

Appendix 1 continued

Monounsaturated Fatty Acids (MUFAs)				
16:1(n-7)	2.18 ± 1.22	2.25 ± 1.40	2.59 ± 2.64	1.34 ± 1.92
17:1(n-7)	0.00 ± 0.00	0.00 ± 0.00	0.41 ± 0.19	0.38 ± 0.22
18:1(n-9)	12.26 ± 7.49	14.83 ± 5.95	14.70 ± 5.11	17.57 ± 6.85
18:1(n-7)	3.47± 1.06	3.68 ± 0.85	3.56 ± 0.94	2.73 ± 1.24
18:1(n-5)	0.00 ± 0.00	0.00 ± 0.00	0.10 0.11	0.05 ± 0.08
20:1(n-3)	0.00 ± 0.00	0.04 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
20:1(n-9)	2.63 ± 2.51	4.04 ± 2.48	4.45 ± 2.09	4.89 ± 2.35
20:1(n-7)	0.02 ± 0.11	0.11 ± 0.20	0.00 ± 0.00	0.00 ± 0.00
22:1(n-11)	0.82 ± 1.42	0.35 ± 1.06	0.00 ± 0.00	0.00 ± 0.00
22:1(n-9)	0.19 ±0.54	1.49 ± 1.70	2.94 ± 2.15	2.47 ± 1.69
24:1(n-9)	0.40 ± 0.58	0.59 ± 0.54	0.00 ± 0.00	0.00 ± 0.00
Polyunsaturated Fatty Acids (PUFAs)				
16:4(n-3)	0.02 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:2(n-6)	0.70 ± 0.40	1.93 ± 5.66	0.00 ± 0.00	0.00 ± 0.00

Appendix 1 continued

18:3(n-6)	0.00 ± 0.00	0.02 ± 0.09	0.02± 0.09	0.00± 0.01
18:3(n-3)	0.04 ± 0.12	0.04 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
18:4(n-3)	0.12 ± 0.26	0.06 ± 0.13	0.43± 0.28	0.21± 0.27
20:2(n-6)	0.04 ± 0.19	0.06 ± 0.10	0.09± 0.11	0.07± 0.11
20:3(n-6)	0.00 ± 0.00	0.00 ± 0.00	0.08± 0.39	0.01± 0.05
20:2(n-9)	0.05 ± 0.23	0.08 ± 0.31	0.00 ± 0.00	0.00 ± 0.00
20:4(n-6)	2.42 ± 0.80	2.15 ± 0.47	2.21± 0.92	1.94± 0.62
20:3(n-3)	0.00 ± 0.00	0.03 ± 0.10	0.10± 0.43	0.01± 0.03
20:4(n-3)	0.20 ± 0.50	1.48 ± 5.57	0.43± 0.28	0.29± 0.30
20:5(n-3)	13.21±7.16	8.85 ± 3.00	12.52± 3.66	9.81± 4.02
22:4(n-6)	0.00 ± 0.00	0.00 ± 0.00	0.05± 0.15	0.01± 0.04
21:5(n-3)	0.46 ± 2.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:3(n-6)	0.00 ± 0.00	0.02 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
22:5(n-6)	0.41 ± 0.28	0.31 ± 0.29	0.38± 0.25	0.21± 0.20
22:5(n-3)	1.71 ± 0.76	1.27 ± 0.73	1.88±0.74	1.27± 0.56
22:6(n-3)	27.99 ± 9.54	27.92±11.59	22.17± 6.83	27.81± 7.93
24:6(n-3)	4.00 ± 7.83	3.66 ± 4.73	0.00 ± 0.00	0.00 ± 0.00

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